STUDIES OF THE MECHANISMS OF DRUG CLEARANCE; WITH SPECIAL REFERENCE TO THE CLEARANCE OF HUMORAL AGENTS.

by

Mohumad Yousufuddin Ansari

M.B., B.S.(Osmania), M.R.C.S., L.R.C.P.(England)

Thesis presented for the Degree of Ph.D.

University of Edinburgh.

October 1942.

Contents.

Among the oxidases, amine-oxidase shows some suggestive peculiarities regarding its susceptibility to enzyme poisons; it is cyanide insensitive, and inhibited by narcotics, but not by reducing agents such as ascorbic acid. It is also inhibited by ephedrine, cocaine and other local anaesthetics. Catechol oxidase, on the other hand, is very sensitive to cyanide, inhibited by ascorbic acid but not by ephedrine or cocaine. Nevertheless most of the findings are in favour of the hypothesis that adrenaline is destroyed in the body by amine-oxidase. But there are some points against it; firstly, that the rate of action of amine oxidase in vitro is too slow to account for the rapid inactivation of adrenaline in the body; secondly, that adrenaline is inactivated in tissues such as rabbit's ears which do not contain amine-oxidase (Gaddum and Kwiatkowski, 1938; and Richter, 1940).

The liver is the chief tissue which contains considerable quantities of amine-oxidase and hepatectomy does not alter the rate of inactivation of adrenaline in dogs (Markowitz and Mann, 1929). Similar objections can be raised against the view that phenol oxidase is responsible for the inactivation/

vation of adrenaline in vivo. Mammalian tissues have been shown to possess very little activity (Duchateau-Bosson and Florkin, 1939). Moreover in some lower animals no relationship was found between the phenolase activity and the distribution of the chromaffin system. For example arthropods which show a high phenolase activity have no chromaffin system, whereas annelida which have a well defined chromaffin system show very little phenolase activity. (Bhagvat and Richter, 1938).

Clark and Raventos (1939) showed that the inactivation of adrenaline by frog's auricle was inhibited by ascorbic acid. This would agree with the view that adrenaline inactivation in the body is due to cytochrome oxidase which is known to be present in all the tissues. De Meio and Luduena (1940) found that the slow inactivation of adrenaline by dog's retractor penis muscle (10-50 per cent. in 2-5 hours) was not inhibited by cyanide (1-2 times 10^{-3} molar), a concentration which would certainly inhibit cytochrome oxidase.

Clark and Raventos (1939) calculated the rate of clearance of adrenaline in the intact cat from the relation between the dosage and duration of action/

action of the drug. They found that for doses of adrenaline below 3 μg/kg. the time of half clearance was constant, but with doses above 3 μg/kg. a constant amount of the drug was destroyed in a unit of time. These results suggest saturation of the inactivating enzyme with doses of adrenaline above 3 μg/kg.

The work here described was an attempt to study the nature of the mode of clearance of adrenaline by continuous infusion of the drug, and the influence of certain enzyme inhibitors on the action and rate of clearance of adrenaline.

The extensive literature on the inactivation of adrenaline has been reviewed and a brief account of the history and chemistry of the drug is given.

ADRENALINE /

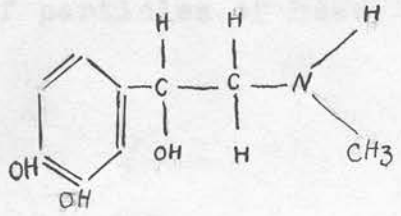
II. ADRENALINE - Historical and Chemistry.

Historical.

Adrenaline was the first of all the hormones to be fully identified chemically. In 1856 Vulpian discovered "chromaffin substance" in the medulla of the suprarenal glands and in association with some other sympathetic cells. The pressor effect of the suprarenal extracts was first demonstrated by Oliver and Schafer (1895), by injecting these extracts intravenously into cats and other mammals. This observation was soon followed by attempts to isolate the active principle of the suprarenal medullary extract. Abel (1898) isolated an impure form which he called epinephrine. Some years later Takamine (1901) and Aldrich (1901) isolated the active principle in a pure crystalline form and named it adrenaline. In 1904 Jowett determined its structure and in the same year the synthesis was accomplished (Stolz, 1904, Dakin, 1904), and later an extensive series of related amines were synthesized (Barger and Jowett, 1905; Barger and Dale, 1910).

Chemistry.

Adrenaline is a white crystalline substance. It is a weak base and forms salts with acids. Chemically it is dihydroxy phenyl ethanol methyl amine, with the structural formula:-



This corresponds to the empirical formula $C_9H_{13}NO_3$ first suggested by Aldrich (1901). Structurally adrenaline is closely related to tyrosine and it is thought that it may be a derivative of this or similar amines present in the body. The laevo-rotatory form of adrenaline is about twelve times more active than the dextro-rotatory form.

III. REVIEW OF THE LITERATURE ON THE INACTIVATION OF ADRENALINE.

A. In vitro.
B. In vivo.

A. In vitro.

Catalytic action of physical and chemical agents.

Adrenaline is readily oxidized in the presence of light and air, though simple boiling does not reduce its activity (Oliver and Schäfer, 1895). The oxidation rate of adrenaline, by hydrogen peroxide and potassium permanganate was shown to reach the maximum at a temperature of 35°C. (Barker et al, 1932). Suprarenal extracts and adrenaline solutions are more rapidly oxidized in alkaline than in acid or neutral solutions (Oliver and Schäfer, 1895; Blix, 1929; Sugawara, 1929; and Barker et al,1932). The presence of particles of heavy metals, such as copper/

copper, iron, calcium, cobalt, nickel, silver, mercury, manganese etc. in solution of adrenaline catalyses the oxidation, copper being the most active (Barker et al, 1932; Schiøld, 1933; Welch, 1934).

Welch (1934) observed that the oxidation of very dilute solutions of adrenaline was catalysed even by coming in contact with the glass surface. Sugawara (1929) studied the rate of oxidation of adrenaline by aeration, in various physiological solutions, including defibrinated blood and distilled water. The approximate times of half inactivation calculated from his results are as follows:

Redistilled water	7½ hours
Sodium chloride solution, 0.85 p.c.	3½ "
Locke's solution	¾-1¼ hours
Tyrode's solution	½ hour
Defibrinated blood	1 hour

These results show that the rate of oxidation of adrenaline in distilled water which does not contain any metallic ions, is very slow. Barker et al (1932) also found that the rate of oxidation of adrenaline/

adrenaline by hydrogen peroxide and potassium permanganate was more rapid in tap water than in distilled water.

Cramer (1911) was very interested by the specificity and rapidity with which formaldehyde destroyed adrenaline in vitro; he found that adrenaline was completely destroyed when 5 c.c. of 1/50,000 solutions was incubated with formaldehyde (5 c.c. 1/500) in 4 to 5 minutes, and in a shorter time with smaller quantities of adrenaline. He suggested that the inactivation of adrenaline in the body was brought about not by a process of oxidation but by combination with metabolic products of cells on which the drug acted. Toscano-Rico and Malafaya Baptista (1935) concluded that the aldehyde formed as an intermediate product of carbohydrate metabolism was responsible for the inactivation of adrenaline in the body.

Inactivation of adrenaline by living tissues.

There is an extensive literature regarding the inactivation of adrenaline by ~~the~~ isolated tissues. Many authors in the late nineteenth and early twentieth century showed that several tissues possessed the power of inactivating adrenaline. Langlois (1897, 1898) found that suprarenal extracts were/

were inactivated when ground with rabbit's liver or intestine, but not with lung. Similar results were obtained by perfusion of adrenaline through intestine and lungs (Elliott, 1905) and through the liver (Livon, 1904; Battelli, 1902). Bain et al (1937) found that adrenaline was completely inactivated after being incubated with liver slices; they noted that this property of liver slices was diminished by dipping them into boiling water, and completely abolished by boiling for two minutes. Geoffridi (1907) investigated the rate of inactivation of adrenaline by perfusing the drug through various organs. He found that the rate of inactivation was most rapid in the liver, moderate in the skeletal muscles and none in the lungs, kidneys or brain. He concluded that the inactivation of adrenaline was a special function of the liver. Tatum (1912) incubated pieces of blood vessels with solutions of epinephrine and found that the solutions markedly lost their potency; while control solutions did not show much diminution. Lawen (1904) found that a considerable amount of epinephrine was lost after repeated perfusions of the solution of the drug through the hind limb of the frog.

Results contradictory to those described above have/

have been described by some authors. Wiltshire (1931) believes that the living tissues do not destroy but protect adrenaline from oxidation in vitro. Embden and Furth (1904) stated that neither perfusion through the organs nor aeration in contact with the pounded tissues caused an appreciable amount of adrenaline to be inactivated. The contradictory results obtained by these authors, however, appear to be due to the enormous quantities of adrenaline (total dose 50 mgm.) used in their perfusion experiments.

Inactivation of adrenaline by blood.

The fact that the activity of suprarenal extracts was not affected by mixing with dog's whole blood was first shown by Oliver and Schäfer (1895) and later confirmed by many other authors (Langlois, 1898; Embden and Furth, 1904). Sugawara (1928-29), however, found that the inactivation of adrenaline by dog's defibrinated blood was stopped after an initial destruction of 60 per cent. in about 2 hours. Bain et al (1936a, 1936b, 1937) showed that adrenaline was destroyed both by the serum and plasma, the rate of destruction being twice as

rapid/

rapid in the former as in the latter. They found that the destruction of adrenaline in both serum and plasma was complete, whereas in whole blood the process stopped after the equilibrium concentration of the drug was reached. They recovered 80 per cent. of the original amount of adrenaline from the equilibrium mixture and showed that the drug in the whole blood was protected by the blood corpuscles; and concluded that blood itself was probably of small significance in determining the inactivation of adrenaline in the body, and that in tissues the conditions for this inactivation were fulfilled.

Inactivation of adrenaline by enzyme systems.

1. Amine-oxidase.

The fact that many tissues inactivate adrenaline in vitro diverted the attention of the investigators towards the isolation of the inactivating substance or substances from various tissues. The work of Blaschko et al (1937a) showed that there was present in the liver, kidneys and small intestine/

intestine of certain animals a cyanide-resistant substance which inactivated adrenaline *in vitro*. These workers incubated cell free extracts of liver, kidneys and small intestine from guinea pigs, rats and rabbits with adrenaline solutions and found that in the presence of oxygen they greatly accelerated the inactivation of the drug. They purified the inactivating enzyme (amine-oxidase) from the extracts and found that, like other enzymes, it was thermolabile and nondialysable. Its action was inhibited by narcotics but not by cyanide and reducing agents, such as ascorbic acid and glutathione. Ephedrine, a sympathomimetic amine, though not affected by amine-oxidase, has been shown to inhibit the inactivation of adrenaline by this enzyme. (Richter and Tingey, 1939). The protective action of ephedrine ~~over~~ against adrenaline oxidation by amine-oxidase has been explained on the theory of substrate competition.

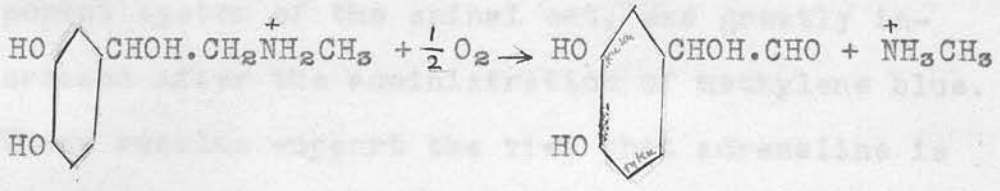
Similar effects were found *in vivo*, and so it was suggested that amine-oxidase was probably the enzyme system that destroyed adrenaline or sympathin in the body (Gaddum and Kwiatkowski, 1938). The amine-oxidase contents of various tissues vary in different species of animals. The livers of omnivorous/

omnivorous animals contain less enzyme than the livers of herbivorous animals; the highest concentration is found in ruminants. Of all the tissues the liver contains the highest amount, while skeletal muscles, kidneys and spleen contain very little and rabbits' ears contain none (Blaschko et al, 1937a, 1937b; Richter and Tingey, 1939; Bhagvat et al, 1939). In addition to adrenaline many other primary and secondary amines are inactivated by amine-oxidase in vitro. Extracts of kidney and liver have been shown by Pugh and Quastel (1937) to inactivate a number of aliphatic amines.

The inactivation of adrenaline and related amines by amine-oxidase, has been shown to depend upon the molecular configuration of these compounds. Blaschko et al (1937b) incubated guinea pig and rat's liver extracts after adding HCN (to exclude the action of other enzymes) with structurally related sympathomimetic amines (adrenalone, adrenaline, p-sympatol, arterenol, epinine, laevo-ephedrine and corbasil). They showed that only those compounds having configuration $= C - CH_2 - N =$ were oxidized by amine-oxidase. Compounds such as carbasil and ephedrine, which do not have this configuration, are not/

not attacked by amine-oxidase. The oxidation of adrenaline by amine-oxidase occurs in the side chain of the molecule; the products of oxidation of amines by amine-oxidase are always an aldehyde and ammonia or a lower amine.

According to Richter (1937) the reaction in the case of adrenaline can be represented by the following equation:-



The relation between the molecular configuration of sympathomimetic amines and their oxidation by amine-oxidase has also been described by Beyer (1941) and Beyer and Vernon (1942). They found that amine-oxidase obtained from the liver of guinea pig, rat or rabbit, oxidized only those phenyl-propylamines which had amino group attached to the terminal carbon atom of the side chain. Beyer and Vernon (1942) have shown that damaging of the liver in the animals by giving carbon tetrachloride or hydrazine prior to administration of compounds, which in vitro are oxidized by amine-oxidase, results in the excretion of these compounds in the same way as those which are not attacked by amine-oxidase, i.e. ephedrine/

enzyme and the chromaffin system in some animals. For example arthropods which do not have a chromaffin system were found to possess the highest phenolase activity. On the other hand lumbricus terrestris and Hirdu medicinalis, which have well defined chromaffin system, showed little phenolase activity. Catechol oxidase is thermolabile and very sensitive to cyanide (Kastle and Loevenhart, 1901). It is inhibited by H_2S, CO, and resorcinol (Richter 1934; Keilin, 1929; Keilin, 1936) but is not inhibited by methylene blue (Philpot, 1937). The oxidation of adrenaline by catechol oxidase is also inhibited by reducing substances, but is comparatively resistant to narcotics and other usual enzyme poisons (Richter, 1934). Keilin and Mann (1938) prepared catechol oxidase from mushrooms and found that it was destroyed by alkalies and acids. They found that the enzyme consisted of the combination of protein and a metal (iron, copper or manganese, etc.), and that the active group of the enzyme always contained copper. Bhagvat and Richter (1938), who worked on the animal phenolases prepared from arthropods and molluscs, found that they/

they oxidized catechol derivatives. They also showed that the apparent activity in a number of arthropods and molluscs was mainly due to haemocyanins and other copper protein complexes, which acted as pseudo-phenolases. They obtained a crystalline copper protein complex from the blood of Cancer Pagus which was catalytically active. Beyer (1941) has shown that amino compounds having one or two hydroxyl groups on the benzene ring are oxidized by phenol oxidase. Bacq (1938) believes that adrenaline is oxidized in the body by catechol oxidase, and that during the oxidation of the drug by this enzyme, a substance with inhibitory properties is produced. Adrenochrome is formed in the course of the oxidation of adrenaline by phenol oxidase, and if this is the process of inactivation of adrenaline in the body, then it should be excreted in the urine in the conjugated form. Richter (1940), however, did not find any increase in the indol derivatives in the urine after oral administration of adrenaline in man.

3. Cytochrome oxidase (indophenol oxidase)

Cytochrome oxidase is known to be present in all tissues (Green and Richter, 1937; Keilin and Hartree, 1938). The best source of this enzyme however/

however is the heart muscle. Cytochrome oxidase is destroyed very rapidly by drying or by treatment with alcohol or acetone (Keilin and Hartree, 1938). Its catalytic activity is completely inhibited by M/100 HCN (Green and Richter, 1937; Keilin and Hartree, 1938), and by H_2S, ~~NaN_3~~ and CO (Keilin and Hartree, 1938). It is not inhibited by methylene blue (Philpot and Cantoni, 1941), and it has a great affinity for oxygen. In vitro it oxidizes adrenaline and a number of catechol derivatives. Adrenochrome is the product of oxidation by cytochrome oxidase. Philpot and Cantoni (1941) estimated the rate of oxidation of adrenaline by liver suspensions and by suspensions of dog's and rabbit's heart muscle. They found that cyanide M/100 completely inhibited the oxidation of adrenaline by heart muscle suspension, and only slightly inhibited the destruction of adrenaline by liver suspension.

Keilin and Hartree (1938) obtained cytochrome oxidase from the heart muscle and found that the oxidation of adrenaline and a number of diamines and polyphenols by the enzyme system was greatly increased by the addition of M/100,000 to M/10,000 cytochrome C. They concluded that cytochrome oxidase/

oxidase does not oxidize adrenaline and other compounds directly but through the cooperation of cytochrome C. Clark and Raventos (1939) showed that isolated frog's auricle inactivated adrenaline and that this action was inhibited by the addition of ascorbic acid (10^{-6}). This observation supports the view that adrenaline in the body is inactivated by cytochrome oxidase which is known to be present in all the tissues. De Meio and Luduena (1940), however, found that the inactivation of adrenaline by dog's retractor penis muscle was not inhibited by cyanide which would have inhibited cytochrome oxidase.

Rate of action of enzymes.

The chief difficulty with regard to the inactivation of adrenaline by enzymes is that the rates of action found have been far slower than the rates of inactivation of adrenaline in the body.

Rate of action of amine-oxidase: Richter and Tingey (1939) incubated liver suspensions containing 100 units/c.c. of amine-oxidase, with adrenaline solutions of 10^{-4} - 10^{-7}, and found that half inactivation occurred in 30 minutes. This time would correspond to half clearance in the liver in/

in 12 mins. and much slower clearance in other tissues which contain relatively little amine-oxidase. With adrenaline concentrations of 10^{-3} only 10 p.c. was destroyed in 30 mins. These rates of destruction are far slower than the rates of inactivation of adrenaline in the body. Kohn (1937) found that amine-oxidase had a much lower affinity constant for adrenaline ($K = 2 \times 10^{-2}$) than for tyramine ($K = 5 \times 10^{-4}$) and so concluded that it was unlikely to be of physiological importance in the clearance of adrenaline. Bacq (1936b) also pointed out that the action of amine-oxidase in vitro takes hours, whilst adrenaline in vivo is inactivated in a few minutes.

Rate of action of phenol oxidase, cytochrome oxidase and peroxidase.

Blaschko and Schlossmann (1940) found with polyphenol oxidase and adrenaline (1:1000) that 70 p.c. was inactivated in 9 mins. at 19°C.; with cytochrome oxidase 75 p.c. inactivation occurred in 51 mins. at 19°C. and with peroxidase at a slower rate than with cytochrome oxidase. The rate of action of polyphenol oxidase approximates to the rate of adrenaline inactivation in vivo.

B. /

B. Adrenaline Clearance in vivo.

Adrenaline disappears from the blood stream very rapidly. Elliott (1905) found that large doses of adrenaline (1 mgm.) injected intravenously into the cat disappeared from the blood in three minutes. Similar results were obtained by De Vos and Kochmann (1905) who injected adrenaline into rabbits and found that the drug disappeared from the blood in about 10 mins. It has also been shown by several authors that the disappearance of adrenaline from the blood stream parallels the effect on the blood pressure (Elliott, 1905; Jackson 1909; Trendelenburg, 1916; Bacq, 1936c); the same is true of many other tissues, such as urethra, uterus, bladder, eye, ileo colic sphincter (Elliott, 1905).

Many workers have shown that intraarterial injections of adrenaline are less effective than the intravenous. Carnot and Josserand (1902) found that moderate doses of adrenaline, injected into the carotid or femoral artery or large doses (0.064 mgm. per kg.) given into the mesenteric artery, produced no effect on the general circulation of dogs. Clark and Raventos (1939) found that the ratio between

the/

the equiactive doses of adrenaline administered into the vein and into the artery was 1:6.

These results can partly be explained by distribution of the drug in the plasma volume, assumed to be 5 p.c. of the body weight. Intravenous injection only mixes with half the blood before it circulates round the body. Hence there is immediate dilution in less than 5 p.c. body weight. Intra-arterial injections, on the other hand, diffuse in the body tissues and mix with the whole blood after first circulation. Adrenaline is quickly distributed in 25 p.c. body weight (tissue fluid volume), hence rapid fall to one fifth of the original concentration is to be expected.

The role of various body tissues in the inactivation of adrenaline in vivo.

Elliott (1905) concluded that adrenaline destruction occurs in all the tissues stimulated by the drug. Many workers, though agreeing with the statement of Elliott, emphasize the importance of the liver in the inactivation of adrenaline in the body. This view is strengthened by the experiments of Beyer (1941) and Beyer and Vernon who found that

some/

some sympathomimetic amines were excreted unchanged when the liver in animals was made inactive by certain poisons. Philpot and Cantoni (1941) found that in vitro methylene blue inhibited amine-oxidase obtained from the liver and had no effect on other oxidases. They compared the equivalent doses of adrenaline given into the jugular and splenic vein before and after the intravenous injection of methylene blue, and found that the ratio of the equivalent jugular and splenic doses was changed from 1:6 to 1:2. after the administration of methylene blue. These results suggest that the reduction in the rate of inactivation of adrenaline after the administration of methylene blue is probably due to inhibition of amine-oxidase in the liver.

Excretion of adrenaline.

Earlier authors (Oliver and Schäfer, 1895; Jackson, 1909) found that the exclusion of the kidneys from the general circulation resulted in no prolongation of the duration of action of adrenaline, and concluded that the drug was not excreted unchanged.

Weinstein and Manning (1937) reported that a protocatechuic acid-like substance was excreted into the urine/

urine after injection of adrenaline in the rabbit, and suggested that this was the oxidation product of adrenaline by amine-oxidase. Richter (1940) however, considers this to be an artifact produced by the action of alkalies on adrenaline. Richter (1940) and Richter and Mackintosh (1941) showed that in man and rabbit, adrenaline administered orally was excreted in the urine in conjugation with sulphates. They recovered 50 to 70 p.c. of the administered adrenaline from the urine, and concluded that conjugation was the main physiological process by which the drug was inactivated in the body.

IV. CUMULATION AND CLEARANCE OF ADRENALINE.

The nature of the clearance of labile drugs such as adrenaline can be judged from the cumulation effect produced by continuous infusion. The two known forms of clearance are:

(a) Clearance of a quantity per minute which is constant and does not change when the amount present in the body is increased. In this case if the quantity cleared per minute is x/kg./min., then continuous infusion with this rate will produce practically no effect, and infusion at a greater rate will result in a steadily increasing response.

(b) Clearance which increases when the amount of drug in the body increases. In this form of clearance, cumulation will occur until the amount introduced per minute equals the amount cleared per minute. Equilibrium or plateau response is thus produced, and when this occurs the rate of clearance can easily be calculated, since it equals the amount introduced per minute divided by the amount cumulated. If a clearance process such as has been described occurs, then a series of different rates of infusion will result in plateaus of cumulation at a series of different levels. Most of the drugs/

drugs, including those whose clearance depends upon enzyme action, are cleared in this manner.

The author (Ansari, 1942) found that the clearance of adrenaline was such that the amount cleared per minute varied as (amount cumulated)$^{0.66}$. Since the publication of this paper a better method for mathematical representation of the result has been devised which differs in some respects from the previous one. The calculations given below are done by this new method.

EXPERIMENTAL.

Methods.

Cats were used in all the experiments. With some exceptions in which chloralose (0.1 kg. intravenously) was given, all the experiments were made on cats whose brain and spinal cord were destroyed under ether anaesthesia. The details are as follows: After weighing, the animal was anaesthetised with ether and placed on an operation table heated by means of electric bulbs underneath. The trachea was exposed by a median incision in the neck and a cannula inserted. The anaesthesia was then continued through the tracheal cannula.

Destruction of the C.N.S.

The head of the cat was firmly held ~~by~~ with the left hand and a vertical incision extending from the vertex to the root of the nose was made in the scalp. At the root of the nose the above incision was joined by a second incision extending to the right ear. A triangular flap of the scalp thus formed was turned up and the periosteum over the exposed bone was scraped off. By means of a trephine an opening was made in the skull. In order to avoid the venous sinus /

sinus (sagittal sinus) the site chosen for trephining was about half an inch to the right of median line. A sharp narrow scalpel was passed through the opening in the skull and the medulla oblongata was cut across at the level of the Foramen Magnum. The brain was then destroyed by means of a curved blunt dissector and the destruction of the spinal cord was completed by passing into the spinal canal through the Foramen Magnum ~~of~~ a flexible smooth tipped copper wire about two feet long. Immediately after the destruction of the brain and spinal cord the opening in the skull was plugged, anaesthesia was discontinued and artificial respiration started.

Ligation and removal of suprarenal glands.

The suprarenal glands were excluded from the general circulation either by their complete removal or by simple ligation in the manner described below.

The abdomen was opened by a median incision and the glands were exposed by incising the overlying peritoneum. Two ligatures one on either side of the gland were tied and to ensure a complete exclusion a third was tied round the body of the gland. The glands in some experiments were removed after/

after such ligation. The ligation as well as the removal of the right suprarenal gland required more care on account of its being in close relation with the inferior vena cava and the liver. After the ligation was completed some warm saline was put in the peritoneal cavity and the abdomen was closed by means of artery forceps.

Removal of the left superior cervical ganglion and section of the vagi.

The incision in the neck made for tracheotomy was enlarged and the left superior cervical ganglion was isolated and removed. Both the vagi were then cut in the middle of the neck.

Blood pressure record: B.P. was recorded from the right femoral artery by means of a mercury manometer. The manometer system contained half saturated sodium sulphate solution. In addition to this, a small quantity of sodium oxalate was put in the arterial cannula as an anticoagulant.

Contraction of the left nictitating membrane. The head of the cat was rigidly fixed in a clamp and the outer corner of the eyelid was incised to allow the free movements of the n.m. A fine thread was then tied in the middle of the n.m. and the contractions were recorded by attaching the free end of the thread/

thread to an isotonic lever with a writing point on a revolving kymograph.

Preparation of adrenaline solution. Adrenaline hydrochloride solution 1:1000, prepared by Parke, Davis and Co., was used in all the experiments. This was diluted with distilled water just before the experiment to make the solutions of the following strengths:

3:10,000
1:10,000
3:100,000
1:100,000
3:1,000,000
1:1,000,000
3:10,000,000
1:10,000,000

These dilutions correspond respectively to 300,100, 30, 10, 3, 1, 0.3 and 0.1 micrograms of adrenaline in 1 c.c. of the solutions.

Fresh diluted adrenaline solutions were made if required after two hours of their preparation.

Administration of adrenaline: The solutions of adrenaline were given into the femoral vein as single injections and continuous infusions. In the case of single injections 1 c.c. of the solution of known strength was taken into a 1 c.c. glass syringe fitted with a fine needle and injected into the/

the femoral vein through the rubber tubing connecting the venous cannula to a burette containing warm saline. In addition another 2 c.c. of warm saline from the burette were run in, making the total volume of the fluid injected to 3 c.c.

Continuous infusions of adrenaline.

The whole success of the experiments with continuous infusions of adrenaline depends upon the maintenance of the uniform rate of inflow of the drug. This was accomplished by means of an apparatus shown in Fig. 1.

This consisted of a 10 c.c. record syringe, a wheel having a circular hole in the centre and a square rod. The wheel was placed between two supporting pillars, and was rotated by means of a belting from an electric motor. The wheel had screw threads in its central hole corresponding to the threads on the square rod. The square rod was passed through the circular hole of the wheel and also through the square holes of the supporting pillars. When the wheel was rotated it made the square rod to rotate also, but the square holes in the supporting pillars prevented this and a forward movement of the rod was resulted. The distance through which/

which the rod was moved with each complete rotation of the wheel was equal to the distance between the threads.

A 10 c.c. syringe filled with adrenaline solution of known concentration was fixed in clamps; its nozzle was connected by means of a rubber tubing to the cannula in the femoral vein, and the plunger was brought in to contact with the square rod which pushed it forward with a uniform rate. The rate of the infusion can be changed either by altering the speed of the motor or by changing the concentration of the drug in the syringe. The latter method was adopted in all the experiments when a change in the continuous dose was desired.

Cumulation with continuous adrenaline infusion.

Oliver and Schäfer (1895) found that intravenous infusions of suprarenal extracts produced a rise in the blood pressure ~~level~~ which maintained its level as long as the infusion was continued. Kretschmer (1907) found that different rates of infusions of adrenaline, in the cat, produced plateau response of different heights. Infusions of larger doses than those which produced the maximum rise of blood pressure, however, did not increase the height of the plateau but prolonged the time of recovery after the infusion was stopped Dragstedt et al (1928) administered adrenaline with uniform rates of 0.2 to 0.4 µg/kg./min. and found that it produced a sustained rise of the blood pressure in normal unanaesthetized dogs. Similarly many other authors have shown that infusions of adrenaline at constant rates will maintain a response at constant plateau for a long time (Trendelenburg, 1924; Rosenblueth, 1932; Prohaska et al (1937).

In figs. (2a, 2b, 2c and 2d) and (3a and 3b) are shown the tracings of the responses of the nictitating membrane and blood pressure, to various doses/

doses of quick injections and continuous infusions of adrenaline in two experiments. The dose of continuous infusions in these experiments ranged from 0.46to 4.66 µg/kg./min. These infusions produced plateaus of different heights according to the dose administered; the height of plateau being below the maximum response of the nictitating membrane or blood pressure. Figs.4a & 4b show the effects of quick injections and of a large dose of continuous infusion (6.02 µg/kg./min.) on the nictitating membrane in another experiment. The height of plateau response in this case also was below the maximum response of the tissue as can be seen from the amplitude of the response to quick adrenaline dose of 86.8 µg/kg. (Fig. 4a).

A study of the response to continuous infusion of adrenaline shows that the plateau is attained in from 2-5 minutes and can be maintained for a long time. The response of the blood pressure to continuous infusions of adrenaline was less satisfactory as the plateau was not maintained, but declined soon after reaching the maximum; this was more marked with large doses of continuous infusion (Fig. 7). The nictitating membrane, on the other hand, gave

a/

a steady plateau response (with large as well as with small continuous dose) which was maintained as long as the infusion was continued. Soon after the discontinuation of the infusion the nictitating membrane and blood pressure began to recover and reached their original level in periods depending upon the rates of infusion and the amount of cumulation.

Estimation of the cumulated amount of adrenaline.

In order to estimate the amount of adrenaline cumulated when a plateau response was attained by continuous infusion, the cats were first standardized by a series of quick injections and the continuous infusions interspersed. The results of 12 experiments, summarized in Tables 1 and 2, are shown in Figs. 5 and 6. The curves in Fig. 5 show the relation between the logarithms of quick and continuous doses of adrenaline and the heights of responses of the nictitating membrane and blood pressure from the collective results of 6 experiments given in Table 1. Fig. 6 shows similar results obtained in 4 sensitive and 2 insensitive cats with the nictitating membrane response.

The/

The sensitivity as is seen from Fig. 6 varied within a fivefold range. The dosage response curves follow a hyperbola, as found by many authors (Rosenblueth, 1932; Bacq, 1936; Clark and Raventos, 1939). The curves in Figs. 5 and 6 are drawn to the formula that expresses the reversible unimolecular reaction:

$$x^{1/k} = \frac{y}{100-y}$$

where x = concentration, y = percentage of response and 100 = the maximum response. The values of the maximum response and k for these curves are given at the foot of each figure. The calculated curves where they deviate from the observed curves are shown by dotted lines. In all cases continuous infusion of adrenaline resulted in a plateau being attained. The graphs show that all the responses to continuous infusions were below the maximum response; hence the plateau was not due to the tissue having contracted to the maximum extent.

In cases where large doses of continuous adrenaline were administered, this point was confirmed by giving a quick injection of the drug in addition to the continuous flow, and this produced an/

an extra response in all cases. In Fig. 7 is shown the response of the nictitating membrane to a continuous dose of 10.1 µg/kg./min., at point "p" an additional quick injection of 12.6 µg/kg. was given, which resulted in an extra contraction of the tissue.

The fact that a plateau effect was produced with large doses (10 µg/kg./min.) indicates that the rate of clearance was increasing with the amounts present as the result of these rates of injection. Hence the conclusion of Clark and Raventos (1939a) that enzyme saturation occurs with doses of over 3 µg/kg. is probably incorrect.

Source of error in the method of estimation of cumulation.

The cumulation with continuous infusion when a plateau is attained has been assumed to be equal to the amount of adrenaline which produces the same effect when given as quick injection. ~~This~~ This assumption will be reasonable if there is no large difference in the distribution in the two cases.

The plasma volume of a cat is about 5 per cent. of the body weight, and the extra cellular fluids are/

are about 25 per cent. of the body weight. A quick injection of adrenaline thus will reach the susceptible tissue before being mixed up with whole blood. Therefore a dose of 1 µg/kg. when injected intravenously will for some time act on the susceptible tissue in a much higher concentration (20 µg/kg.). In the case of continuous infusion, however, there is enough ~~of~~ time for the equal distribution in the blood and tissue fluid; so a dose of 1 µg/kg. is unlikely to produce a concentration of more than 4 µg/kg.

In order to determine how far this source of error has affected the results, a series of doses of adrenaline were given alternately as quickly as possible and spread over more than a minute. The responses of the blood pressure were equal under the two conditions, whilst the responses of the nictitating membrane were about 25 per cent. less with the slow injection (Table 3). These results indicate that the ratio of the equiactive doses with slow and quick injection is certainly less than 2:1 and may be equal to 1:1.

The responses of the nictitating membrane and blood pressure to quick injection of adrenaline take from 0.5 to 3 minutes to reach the peak, whilst

the/

the response to continuous infusion attains a plateau in about 3-5 minutes. Adrenaline therefore does not act very rapidly but quickly attains a plateau with continuous infusion. The above facts indicate that the differences in distribution with the two forms of injections of adrenaline (quick and continuous) are not very important; hence the assumption that the height of plateau produced by continuous infusion indicates the concentration of adrenaline, equal to the amount which produces the same effect given as quick injection seems to be reasonable.

The blood pressure responses were not as satisfactory for measurement as were those of the nictitating membrane, but in general showed the same relation between dosage and height of plateau response. The ratio quick dose (μg/kg.)/ continuous dose (μg/kg./min.) was nearly unity with doses near the threshold and rose to about 5 with large doses.

Relation between continuous and quick dosage.

Figs. 8 and 9 show the averages of the results of 27 and 16 experiments given in Tables 4 and 5. The relation between these two forms of dosage is that/

that continuous dose varies as (quick dose)$^{2/3}$. The amount introduced per minute when the plateau is attained equals the amount removed per minute, the quick dose indicates the amount cumulated. Hence the figures show the relation between the concentration of the drug present and the amount removed per minute. The relation found indicates therefore a clearance by a process in which the amount of substrate changed per minute (i.e. rate of introduction b) varies as (substrate concentration)$^{2/3}$ or $b = k\,(x)^{2/3}$, where x is the concentration of adrenaline.

$$\frac{dx}{dt} = -k.x^{2/3}$$

$$x^{-2/3}\,dx = -kdt$$

Integrating we get

$$x^{1/3} = x_o{}^{1/3} - \frac{k}{3}t$$

where x_o = concentration at zero time.

Dosage duration relation

If the above equation expresses the clearance then the relation between the concentration x_o at zero time and the duration of action (t) measured till x falls to a threshold value of x_1 will be as follows:

$$x_o{}^{1/3} = x_1{}^{1/3} + \frac{k}{3}t$$

This/

This implies that t shows a linear relation to $x_o^{1/3}$. This can be seen from Figs. 10 and 11, which give the averages of the results of 8 experiments (Tables 6 and 7).

Analysis of the response to cumulative dosage.

In Fig. 12 is shown the response of the nictitating membrane to a continuous dose of 3.4 µg/kg./min. adrenaline administered for 10 mins. The scale on the right ordinate shows the adrenaline equivalent (µg/kg.) calculated from the dosage-response curve. The plateau height indicates cumulation of 14 µg/kg. Curve A in the figure shows the response which should occur if there were no clearance. Curve B shows the cumulation of adrenaline calculated by means of the following formula derived from the relation found between quick and continuous dosage described before.

$$\frac{dx}{dt} = b - kx^{2/3}$$

or $$\frac{1}{b - kx^{2/3}}\, dx = dt$$

where x is the concentration of adrenaline and (b) the rate of introduction of adrenaline.

The value of k can be found from the graph (Fig.13) which shows the relation between $x^{1/3}$ and/

and time (t) and equals 0.39. The times (t) for different values of concentration (x) were calculated by integrating the expression $\int_b \frac{dx}{b - kx^{2/3}}$ graphically. According to this calculation the concentration rises to 17 µg/kg. in 10 minutes.

Curve C, which shows the course of clearance when the initial concentration is 17 µg/kg., was calculated by means of the formula:

$$x^{1/3} = x_o^{1/3} - \frac{k}{3} t$$

$x_o^{1/3}$ = original concentration = $17^{1/3}$

= 2.57

k (from graph 13) = 0.39

Hence $t = \frac{2.57 - x^{1/3}}{0.13}$

By putting the values of $x^{1/3}$ the time for these concentrations can easily be calculated.from the above formula.

Fig. 14 shows the analysis of the response of the nictitating membrane to a large dose of adrenaline (6.02 µg//kg./min.) The method of calculation was the same as that employed for the analysis of Fig. 12. Fig. 15 shows the relation between dose$^{1/3}$ and the duration of action (t) found in the experiment, and from which the values of

k/

k in the equation for cumulation and clearance were found.

It is seen in these figures that the calculated cumulation and recovery curves are in approximate agreement both as regards the concentration attained in given time of infusion and as regards the duration of the clearance. The differences between the observed and theoretical curves may be perhaps due to the mechanical properties of the plain muscle of the nictitating membrane.

Additive effects.

The validity of the method used depends upon the assumption that the production of a plateau response of a certain height represents that a certain concentration of adrenaline is present in the blood stream.

This assumption was tested by giving continuous infusions until plateaus were attained and then adding a quick injection of adrenaline. Figs. 16a, 16b and 16c show the responses of the blood pressure and nictitating membrane to quick adrenaline injection and also the effect of a quick dose of 11.5 µg/kg. adrenaline administered during continuous infusions of the drug. It can be seen that the quick dose of 11.5/

11.5 µg/kg. when given during the continuous infusion of 0.53 or 1.79 µg/kg./min. produced about the same height of response as when given alone. But with the continuous dose of 5.3 µg/kg./min. the response to quick injection was decreased. The results with the nictitating membrane from this and another similar experiment are shown in Figs. 17 and 18 respectively. The continuous line and dots represent the relation between dosage and height of response of the nictitating membrane to quick injections of adrenaline. The crosses show the heights of plateau produced by continuous infusions at the rates indicated by the abscissae.

The dotted lines, circles and numbers indicate the extra response produced by quick injections of adrenaline given during the continuous infusions. The adrenaline equivalents to the crosses and circles can be read off from the dosage-response curve. Table 8 shows how the effect of the additional quick adrenaline injection was calculated from the results given in Fig. 18.

With low doses of adrenaline the difference between the adrenaline equivalents of the responses is similar to the additional quick dose of adrenaline that was given, but with larger doses the calculated dose/

dose was 3 to 5 times as great as the dose given Table 9 shows the figures calculated in a similar manner from 6 experiments with continuous doses greater than 5 μg/kg./min. which produce a response of one-quarter the maximum or less, the figures calculated from the response to additional injection are approximately correct, but when large doses were given with continuous injection the added response was much greater than was ~~to be~~ expected.

Blood pressure responses also were measured and these gave similar results with low rates of continuous infusion, but with higher rates the plateau was not maintained at a constant level, and hence quantitative estimates of additional response could not be made.

Interpretation.

The most probable reason for the excessive responses produced by quick doses after large continuous doses is that the latter saturate the tissue fluid and cells with adrenaline and hence check the diffusion out of the plasma of quick doses.

In calculation of the effect of additional quick/

quick doses of adrenaline described above, the fact that the dosage-response curve follows a hyperbola will lead to quite different conclusions unless caution is exercised. For example the relation between the rate of continuous infusion and the amplitude of the nictitating membrane response to a constant dose of added quick injection found from Fig. 18 are as follows:

Continuous injection µg/kg./min.	0	3.4	5.0	6.8	10
Height of response (n.m.) to 10.2 µg/kg.	42	34	26	22	13
Adrenaline equivalent calculated from dosage response curve	10	10	31	42	50

This shows that as the continuous dose increases, the amplitude of the nictitating membrane response to a constant quick dose is decreased, but if the adrenaline equivalents of these responses be measured from dosage-response curve, it is found that this figure rises as the rate of continuous injection is increased.

It is evident from these results that the measurement of the amplitude of response without considering the dosage-response relation, would lead/

lead to the wrong conclusion that continuous adrenaline infusion inhibited the action of quick injections of the drug and this might be produced as a proof of the "potential action" of adrenaline. In fact the reverse is the truth and continuous administration increases the effects produced by quick injection. This provides a simple example of the errors that can occur in calculating synergisms or antagonisms of drug action.

V. THE INFLUENCE OF ENZYME INHIBITORS ON THE ACTION AND CLEARANCE OF ADRENALINE.

Enzymes differ in their behaviour towards different enzyme poisons; for example amine-oxidase is inhibited by narcotics, cocaine and ephedrine, but not by ascorbic acid or cyanide.. Phenol oxidase and cytochrome oxidase, on the other hand, are inhibited by cyanide and ascorbic acid but not by cocaine or ephedrine.

The nature of the enzyme system concerned in the inactivation of adrenaline in the body therefore can be studied by testing the influence of certain enzyme inhibitors on the action and rate of clearance of the drug in vivo.

Experiments here described were made in order to study the influence of cocaine, pyrogallol and ascorbic acid on adrenaline clearance in the cat and in the light of this probable mechanisms of their action discussed.

Methods. In general the methods used were the same as have been described before. The brain and/

and spinal cord of the cat were destroyed under ether anaesthesia. The suprarenal glands were excluded from the general circulation by ligation, the vagi were cut and the left superior cervical ganglion was removed as the left nictitating membrane was used in all the experiments. The blood pressure was taken from the femoral artery. The response of the nictitating membrane was recorded by an isotonic lever. All the drugs were injected into the femoral vein.

The response of the nictitating membrane to continuous infusion of adrenaline gave more satisfactory results than that of the blood pressure, as with the latter the plateau effect was not maintained for any length of time but declined as the infusion was continued.

(1) The influence of pyrogallol.

Pyrogallol is a strong reducing agent. It prevents the oxidation of adrenaline in vitro. Bacq (1936b,c) showed that various reducing agents, and pyrogallol in particular, greatly increased the duration of action of adrenaline. He concluded that they did not prolong the/

the time during which adrenaline remained in the blood stream, but decreased its rate of destruction in the tissues. He also found that pyrogallol produced a tenfold increase in the amplitude of the response of the nictitating membrane to adrenaline. Clark and Raventos (1939) found that the rate of clearance of adrenaline was reduced to one fourth after the administration of pyrogallol. They did not observe any marked increase in the amplitude of the response of the nictitating membrane or blood pressure.

EXPERIMENTAL.

In the present experiments the response of the nictitating membrane and blood pressure both to quick injections and continuous infusions of adrenaline were first determined. Pyrogallol in doses of about 40 mg/kg. was dissolved in distilled water and slowly injected into the femoral vein. After an interval of about 5 minutes, the quick and continuous adrenaline injections were then repeated; the doses of the drug used were the same as before the administration of pyrogallol.

In Fig. 19 the logarithm of the dose of adrenaline/

adrenaline given both quickly and continuously is plotted against the height of the response of the nictitating membrane from the collective results of four experiments given in Table 10.

The curves A and B in the figure represent the effect of quick adrenaline injections before and after pyrogallol respectively. It can be seen that no marked increase in the response of the nictitating membrane to threshold doses of adrenaline occurs after pyrogallol. With large doses, however, there was an increase in the amplitude after pyrogallol, the doses for equivalent effects being decreased threefold.

The relation between continuous dose and the height of plateau response before and after pyrogallol are shown by lines A_1 and B_1 in Fig. 19. The equivalent to any plateau height, i.e. the amount that has cumulated, can be read off from curves A and B. In Fig. 20 the continuous dose of adrenaline is plotted against the equivalent quick dose, both before and after pyrogallol, from the averages of the results of 8 experiments. given in Table 11. It can be seen that pyrogallol reduced the rate of inactivation of adrenaline, so that/

that the amount of equivalent quick dose to a certain continuous dose was higher than before its administration, but it did not change the relation between these two forms of dosage, i.e. "continuous dose varies as (quick dose)$^{2/3}$.

Dosage duration relation before and after pyrogallol.

In Fig. 21 the cube root of the dose of adrenaline is plotted against the duration of action of the drug on the nictitating membrane from the averages of the results of 7 experiments (Table 12). Line B in the graph shows the relation found before and A after the administration of pyrogallol. The difference between the rates of clearance of adrenaline found from the slope of these curves indicates that the clearance of adrenaline is reduced to about 40 per cent.

Response to continuous infusion of adrenaline after pyrogallol.

Fig. 22 represents the response of the nictitating membrane to a continuous dose of 1.09 µg/kg./min. given for 11.5 mins. after the administration of 40 mg/kg. of pyrogallol. The right ordinate in the figure shows the adrenaline equivalent (µg./kg.) for various heights of response/

response calculated from dosage response curve. The height of the plateau indicates cumulation of 5.62 μg/kg. adrenaline.

Curves A and B in the figure show the course of cumulation and clearance calculated by means of the formulae:

$$\frac{1}{b - kx^{2/3}}\,dx = dt \quad \text{for cumulation}$$

$$x^{1/3} = x_o^{1/3} - \frac{k}{3}\,t \quad \text{for clearance}$$

derived from the relation between quick and continuous dosage. ($b = k(x)^{2/3}$ where b = the continuous dose and x the concentration). The value of k/3 being found from Fig. 23 which shows the relation between the $(\text{dose})^{1/3}$ and the duration of action. The calculated cumulation and clearance are in approximation with observed course of cumulation and clearance.

In Fig. 24 are shown the responses of the nictitating membrane to continuous dose of 1.48 μg/kg./min. adrenaline infused (A) before and (A_1) after pyrogallol.administration. The scales on the right ordinate show the adrenaline equivalents (μg/kg.) (1) before and (2) after pyrogallol, and

are/

are calculated from the dosage response curves. Curves B and B_1 represent the cumulation and C and C_1 the clearance before and after pyrogallol, calculated in the same manner as shown for Fig.23. The values of $\frac{k}{3}$ was found from the curves given in Fig. 25 which shows the relation between $(\text{dose})^{1/3}$ and duration of action before and after pyrogallol administration.

Comparison of the responses of the nictitating membrane in Fig. 24 shows that the increase in the height of plateau produced by pyrogallol is due to a higher degree of cumulation of adrenaline. The greater amount of adrenaline cumulation after pyrogallol may be due to a diminution in the rate of its clearance.

(2) The Influence of Cocaine.

Chemistry. Cocaine is obtained from the leaves of erythroxylon coca. It is a white crystalline substance not easily soluble in water but soluble in organic solvents and destroyed by prolonged boiling. It is a strong base and forms salts with acids, the hydrochloride being most frequently used in medicine. Cocaine is double ester of ecgonine and benzoic acid and methyl alcohol. Its formula has been proved by the synthesis of cocaine from these constituents.

It has long been known that cocaine potentiates the action of adrenaline *in vivo* as well as *in vitro*. Frohlich and Loewi (1910) found that in the intact animal cocaine augmented the action of adrenaline on blood pressure, pupil, and urinary bladder. Later many authors showed that the action of adrenaline on the isolated tissues and the tissues in situ was potentiated by cocaine. Thienes and Hockett (1928) reported augmentation of adrenaline effect on the isolated uterus of the virgin rabbit. Tripod (1940) and Jang (1940) found similar augmentation of adrenaline action on the isolated frog's and cat's heart, rabbit's ear, virgin cat's uterus and intestine in the presence of low concentrations of cocaine. Tatum (1920) found that in dogs and rabbits the response of the blood pressure to splanchnic stimulation was increased after intravenous administration of small doses of cocaine. Tainter (1929, 1930, 1931) and Wirt and Tainter (1932) found that the blood pressure response to adrenaline was doubled after subcutaneous injection of 15-30 mg./kg. of cocaine/

cocaine hydrochloride. Bacq and Fredricq (1935) showed that the response of the nictitating membrane to adrenaline was increased ~~by~~ sixfold after cocaine administration. Luduena (1940) found with some sympathomimetic amines (epinine and sympatol) that their effect on the dog's retractor penis muscle and blood pressure was potentiated by intravenous cocaine in doses of 0.5 to 4.5 mg./kg.

Rosenblueth (1931) observed augmentation of adrenaline effect in the nonpregnant uterus and on the stomach of the cat with cocaine. He also (1932) suggested that cocaine produced an increase in the duration of action of adrenaline by lowering its threshold dose. Labate (1941) found that the inhibitory effect of adrenaline on the uterus of the nonpregnant cat lasted two or three times longer, when 0.3 mg. cocaine was given immediately after the injection of adrenaline. Clark and Raventos (1939a) concluded from their experiments on the action of adrenaline and cocaine on the blood pressure and nictitating membrane of cats, that the increase in the duration of action after cocaine was due to the change in the threshold doses. Tainter(1930) concluded that the potentiating action of cocaine

was/

was not due to any interference with the oxidation processes in the tissues.

Lawrence et al.(1942) calculated the rate of inactivation of adrenaline in Locke's solution perfused through the hind limbs of the cat. They found that the presence of cocaine in the perfusion fluid in concentrations of 1:500,000 to 1:150,000 caused a 40 per cent. decrease in the rate of inactivation of adrenaline.

The influence of cocaine and other local anaesthetics on the inactivation of adrenaline by enzymes in vitro has recently been studied by the following investigators: Philpot (1940) showed that the rate of oxidation of adrenaline in vitro by amine-oxidase obtained from the guinea pig's liver was reduced 60 to 100 per cent. by cocaine, percaine and stovaine. This author has also shown that cytochrome oxidase is also inhibited by cocaine. Bayer and Wense (1938) and Wense (1939) showed that cocaine slowed the rate of oxidation of adrenaline by tyrosinase and acetaldehyde. Bain et al. (1937), on the other hand, could not find any change in the rate of destruction of adrenaline/

adrenaline incubated with liver slices after the addition of cocaine.

EXPERIMENTAL.

Cocaine hydrochloride dissolved in distilled water was injected intravenously in doses of 1.5 to 10 mg./kg. In the majority of animals this alone gave rise to a response in the nictitating membrane and blood pressure. The dose of cocaine above 3 mg./kg. always produced a response. The response of the nictitating membrane to cocaine developed slowly, reached a peak in about 7 mins. and took a long time for recovery. An example of such a response is shown in Fig. 26.

Intravenous doses of cocaine from 1.5 to 2 mg./kg. increased the sensitivity of the nictitating membrane to small as well as to large doses of adrenaline (0.03 to 50 µg/kg.)(Table 13). The doses of cocaine above 3 mg./kg. were generally found to inhibit the action of large doses of adrenaline (above 15 µg/kg.). Fig. 27 shows the effect of cocaine on the responses of the nictitating membrane to various doses of adrenaline in two experiments. Curves B and B_1 show the relation/

relation between the amplitude of the response and the dose of adrenaline before and after the administration of 3.9 mg/kg. of cocaine respectively. It can be seen that responses of the nictitating membrane to adrenaline doses above 15 μg/kg. were diminished. The antagonising effect of cocaine was more marked after a larger dose and can be seen from the results of another experiment shown by curves A and A_1 in the figure.

The effects of sensitizing doses of cocaine on the action and clearance of adrenaline.

As for pyrogallol, the cats were first standarized to single injections of adrenaline and this was followed by continuous infusion of the drug. Then cocaine hydrochloride dissolved in distilled water was injected through the cannula in the femmoral vein. 10-15 minutes were then allowed to elapse, for the cocaine to be distributed throughout the body. Single injections and continuous infusions of adrenaline were then repeated in the same doses as before the administration of cocaine.

Fig. 28 (Table 14) gives relations between the/

the amplitude of the response of the nictitating membrane to single injections and continuous infusions before and after cocaine administration. These results show that cocaine produces a marked lowering of the threshold dose of adrenaline. The minimum effective dose of adrenaline was reduced from 0.3 to 0.03 µg/kg. after cocaine injection. It can also be seen that the proportional increase in the sensitivity to adrenaline was larger when tested with doses of adrenaline below 1 µg/kg. than when tested with larger doses. The effect of cocaine on the blood pressure responses to adrenaline both when given as quick injections or continuous infusion gave similar results to that of n.m. (Fig. 29 and Table 15).

Relation between quick and continuous dosage after cocaine.

The plateau effect produced by continuous infusion of adrenaline after cocaine administration was limited to the rates of about 3 µg/kg./min. corresponding to the cumulation of about 30 µg/kg. Beyond this dose no definite plateau response was obtained. The amount of concentration reached as a result of continuous infusion was estimated from the/

the equivalent quick dose as before. The relation between these two forms of dosage after cocaine administration is shown in Fig. 30 by line A from the averages of 5 experiments given in Table 16. The graph shows that the relation found (continuous dose varies as (quick dose)$^{\frac{2}{3}}$ does not change after the administration of cocaine. The above relation indicates a form of clearance, the course of which follows according to the formula $x^{\frac{1}{3}} = x_0^{\frac{1}{3}} - \frac{kt}{3}$ In such a case the relation between (dose)$^{\frac{1}{3}}$ and the duration of action of adrenaline should be linear, as is shown by the graph in Fig. 31 (Table 17).

The rate of clearance of adrenaline before and after the administration of cocaine can be calculated from the slope of the curves B and A respectively. Comparison of these rates shows that cocaine has reduced the rate of clearance of adrenaline to 55 per cent.

(3) /

(3) Influence of Ascorbic Acid. $C_6H_8O_6$.

Ascorbic acid is a strong reducing substance. It oxidizes itself in the air, particularly when it is in the form of salt, or in a feebly alkaline solution; but at pH 7 only if some oxidative catalyst be present, such as copper. It is more stable in a faintly acid medium.

Ascorbic acid is well known to act as a stabiliser of adrenaline in vitro by virtue of its reducing property. Bacq (1936 a) found that the oxidation of adrenaline in vitro was inhibited by ascorbic acid in concentrations of 0.01 to 0.1 per cent. Szent Gyorgyi (1928) showed that ascorbic acid, in a concentration of 0.0025 per cent., was sufficient to delay in vitro oxidation of adrenaline by the peroxidase system.

The destruction of adrenaline by isolated tissues has also been found to be influenced by ascorbic acid. Clark and Raventos (1939) calculated that the inactivation of adrenaline by auricular strips was reduced to one fourth by the addition of ascorbic acid in a concentration of 0.0001 per cent. (10^{-6}).

The/

The potentiation of adrenaline action has also been demonstrated by Schupfer (1940). He found that the mydriatic action of adrenaline on the isolated frog's eye and the isolated iris of the ox was increased after the addition of ascorbic acid in a concentration of 0.05 per cent. The maximum effects were obtained at 0.1 per cent. concentration of ascorbic acid. Welch (1934) concluded that reducing substances such as glutathione and ascorbic acid normally present in the body, are in sufficient concentration to prevent the destruction of adrenaline from direct oxidation by molecular oxygen, but they do not prevent its destruction by enzymes in the animal organism. The results obtained *in vitro* by various investigators, indicates that an auto-oxidation, destruction by enzymes, and the inactivation by the isolated tissues are prevented by ascorbic acid. On the other hand, the destruction of adrenaline *in vivo* is not affected by ascorbic acid at all.

Bacq (1936 a) and later Clark and Raventos (1939) found that the intravenous injection of ascorbic acid neither prolonged the duration nor increased the amplitude of the response of n.m.

or/

or blood pressure to adrenaline. The former author suggested that the ineffectiveness of ascorbic acid was probably due to its rapid oxidation in the body.

In the following experiments the effects of large doses of ascorbic acid given as single injection or continuous infusions on the action and clearance of adrenaline have been investigated. In addition, the ascorbic acid content of the n.m. was estimated chemically in order to find out whether the injection of ascorbic acid increased its content in the n.m. at the time of the experiment.

Cats were prepared as for the previous experiments and the methods employed were the same as for pyrogallol and cocaine. Single injections of adrenaline in increasing doses were first given and the responses of the n.m. and blood pressure were recorded. This was followed by continuous infusions of adrenaline. Ascorbic acid (neutralized to pH 4) was either given as a single injection (about 1 gram per kg.) or continuous infusions (20 to 40 mg./kg./min.). In cases

of continuous infusions of ascorbic acid, the adrenaline injections were not started for about 4 minutes. These continuous ascorbic acid infusions were kept running until the effect of the adrenaline injected was over.

Fig. 32 shows that ascorbic acid caused no measurable change in the height of the response to either single or continuous adrenaline injections. Neither was the duration of the response of the blood pressure or n.m. to single adrenaline injections affected (Fig. 33).

In Fig. 34a and 34b are shown the tracings of the responses of the n.m. and blood pressure to a continuous dose of 5.53 µg/kg./min. adrenaline (A) before ascorbic acid, (B) simultaneously with a continuous dose of about 38 mg/kg./min. ascorbic acid. It can be seen that the height of the plateau response and time required for recovery when adrenaline is stopped are practically the same before and with ascorbic acid injection. The ascorbic/

ascorbic acid content of the n.m. was 0.1 mg. per 100 mg., whereas the n.m. of the other side removed before ascorbic acid injection contained 0.011 mg. per 100 mg. of ascorbic acid.

Estimation of ascorbic acid in the nictitating membrane of the cat.

The method used was the modification of the micro-method for estimation of ascorbic acid in the blood described by Farmer and Abt (1936). The n.m. was removed, weighed on the torsion balance, cut into small pieces and transferred to a test tube containing 5 c.c. of 10 per cent. acetic acid. This was then ground and centrifuged. To 2 c.c. of the supernatant fluid,2 c.c. of freshly prepared 5 per cent. metaphosphoric acid was added. This was again centrifuged and 2 c.c. of supernatant fluid were titrated against dichlorphenol indophenol (2 tablets, each tablet of the titration value equivalent to 1 mg. of ascorbic acid). The dichlor-indophenol was run in from a Conway burette into the solution and then kept stirred by a stream of carbon dioxide. A blank titration in metaphosphoric acid and water was done and allowance was made for it in the calculation.

In/

In the case of the n.m. after administration of ascorbic acid, the solution was diluted again, with metaphosphoric acid before the titration and allowance was made in the calculation. The results of 9 experiments are given in Table 18. The normal quantity of ascorbic acid found was about 0.01 mg. per 100 mg. The high values in the preliminary experiments nos. 4 and 5 are obviously due to some experimental errors. The table 18 shows that the single injections or continuous infusions of ascorbic acid produce an increase in the quantity of ascorbic acid in the n.m. depending on the quantity administered.

The maximum concentration found as the result of ascorbic acid injection was 0.15 mg. per 100 mg. Control tests were made to exclude the possible effects of adrenaline alone on the ascorbic acid content of the n.m. This shows that adrenaline has no effect on the reducing agent content (ascorbic acid) of the n.m.

VI. DISCUSSION.

The results of the experiments show that the height and the duration of the nictitating membrane and blood pressure responses to adrenaline are increased by cocaine. The responses of the nictitating membrane to adrenaline in doses above 15 mg./kg. are, however, diminished instead of being increased when doses of cocaine exceeded 3 mg/kg.

Burn and Tainter (1931) observed that the inhibition of the uterus of the virgin cat caused by adrenaline was reduced by cocaine. Macgregor (1939) found that low concentrations of cocaine potentiated whereas high concentrations inhibited the action of adrenaline on the isolated uterus of the cat. More recently Tripod (1940) showed that local anaesthetics, namely, butyne, percaine and stovaine, possessed sympathomimetic action, and like cocaine, were capable of modifying the action of adrenaline either by potentiating or by inhibiting its action.

Curtis (1929) found that ephedrine when given/

given in large doses, diminished the blood pressure response to adrenaline in the chloralosed cat. Later Gaddum and Kwiatkowski (1938) showed that ephedrine in small doses potentiated the nictitating membrane response to adrenaline and to the nerve stimulation. They advanced a theory to explain this ephedrine and adrenaline potentiation and antagonism. According to this hypothesis, ephedrine augments the action, by attaching itself to the molecules of the enzyme which destroys adrenaline. The inhibition on the other hand is brought about by the attachment of ephedrine to the sympathetic receptors of the cells, which makes them inaccessible to adrenaline.

Cocaine is not chemically related to sympathomimetic amines, but it has a weak sympathomimetic action like ephedrine. By virtue of this property it may augment or inhibit the action of adrenaline in a manner similar to that of ephedrine as described by Gaddum and Kwiatkowski (1938). The reduction in the rate of inactivation can be accounted for as due to the inhibition of the enzyme concerned. The rate of clearance of adrenaline was reduced to 55 per cent. after cocaine and to about 40 per cent. after pyrogallol. These drugs/

drugs, however, did not disturb the relation found, i.e. the continuous dose varies as (quick dose)$^{2}/3$. The maximum dose of continuous adrenaline and the corresponding amount of cumulation which produced plateau effect were found to be reduced after cocaine or pyrogallol administration. This suggests that after pyrogallol and cocaine enzyme saturation takes place earlier than in the absence of these drugs; this is probably due to the inhibition of the enzyme by these two drugs. Bacq (1936 a) found that ascorbic acid neither prolonged nor intensified the action of adrenaline in vivo. He ascribed the failure of ascorbic acid to influence the action of adrenaline to its rapid oxidation in the body. In order to verify this explanation of Bacq (1936 a), the quantitative estimations of ascorbic acid in the nictitating membrane of the cat before and after its administration were made. The results show (Table 18) that ascorbic acid in the reduced form increased in quantity in the nictitating membrane as the result of its administration and that it is not oxidized as rapidly as suggested by Bacq.

The destruction of adrenaline through various agencies/

agencies *in vitro* (by auto oxidation, destruction by enzymes (peroxidase) and inactivation by various isolated tissues) has been shown by many investigators (Bacq, 1936a; Szent Gyorgyi, 1928; Clark and Raventos, 1939; and Schupfer, 1940) to be inhibited by ascorbic acid in concentrations ranging from 0.0001 to 0.1 per cent.

As is seen in the present experiments ascorbic acid does not seem to have any inhibitory effect on the inactivation of adrenaline *in vivo*, even when its concentration in the tissue tested (nictitating membrane) is raised to the limit (0.15 per cent.) which is sufficient to be effective *in vitro*. It seems reasonable therefore to conclude that adrenaline is probably inactivated *in vivo* by some enzyme system which is not affected by ascorbic acid. This supports the view that adrenaline inactivation *in vivo* is brought about by amine-oxidase, the action of which *in vitro* is not inhibited by ascorbic acid.

But recently Richter (1940) and Richter and Mackintosh (1941) have shown that adrenaline administered by mouth in man and rabbits is inactivated in the body by the process of conjugation with sulphate, and about 70% of adrenaline in this form/

form is excreted in the urine. The enzyme system taking part in such a process (sulphosynthase) appears to be inhibited by cocaine and pyrogallol but not by ascorbic acid. The inactivation of adrenaline in vitro by some isolated tissues, however, seems to be a different process from that in vivo, as it is inhibited by ascorbic acid.

VII. SUMMARY

1. Continuous infusion of adrenaline in the cat produces over a wide range of doses a plateau response both in the nictitating membrane and blood pressure.

2. This indicates that enzyme saturation does not occur even with large doses (correction of conclusion of Clark and Raventos, 1939a).

3. The relation between the continuous and equivalent quick dosage showed that the amount cleared per minute varied as (concentration present)$^{2/3}$.

4. Such a form of clearance was also found by dosage-duration relation as it showed a linear relation between (dose)$^{1/3}$ and the duration of action.

5. The mathematical calculations of the course of cumulation and clearance of adrenaline made by means of the formulae derived from the above relation approximate with the observed results.

6. Mathematical methods calculating the clearance of adrenaline from its pharmacological effects on the/

the blood pressure and nictitating membrane of cat have been used to study the effects of drugs which inhibit its destruction.

7. The rate of clearance of adrenaline calculated was diminished to about 40 per cent. by pyrogallol and to 55 per cent. by cocaine, but was not affected by ascorbic acid.

8. Large doses of cocaine inhibited the effect of large doses of adrenaline on the nictitating membrane.

9. None of the enzyme inhibitors tested altered the relation found, i.e. continuous dose of adrenaline varies as (quick dose)$^{2/3}$.

10. The failure of ascorbic acid to prolong the action of adrenaline was not due to its destruction, since its presence in the tissues in adequate concentration (0.15 per cent.) was demonstrated chemically.

Acknowledgments.

I am greatly indebted to the late Professor A.J.Clark, under whose kind guidance the major part of this work was carried out. I also wish to thank Professor J.H. Gaddum amd Dr J.M. Robson for their help and suggestions during the course of this research. The expenses for the animals and drugs were defrayed by a grant from the Moray Research Fund of the University of Edinburgh, for which I express my thanks.

VIII. BIBLIOGRAPHY.

Abel, J.J. (1897-98). Johns Hopkins Hosp. Bull. 9,215.

Aldrich, T.B. (1901). Amer. J. Physiol. 5,457.

Ansari, M.Y. (1942). Quart. J. exp. Physiol. 31,161.

Bacq, Z.M. (1936a). Arch. Int. Physiol. 42, 340.

Idem. (1936b). J. Physiol. 87, 87P.

Idem. (1936c). Arch. Int. Physiol. 44, 15.

Idem. (1938). J. Physiol. 92, 28P.

Bacq, Z.M. and Fredricq. (1935). Arch. Int. Physiol. 40,297.

Bain, W.A., Gaunt, W.E. and Suffolk, S.F. (1936a). J. Physiol. 87, 8P.

Bain, W.A. and Suffolk, S.F. (1936b) Ibid. 86, 34P.

Bain, W.A., Gaunt, W.E. and Suffolk, S.F. (1937). J. Physiol. 91, 233.

Barger, G. and Dale, H.H. (1910). Ibid. 41,19.

Barger, G. and Jowett (1905). Trans. Chem. Soc. 87, 967.

Barker, J.H., Eastland , C.J. and Evers, N. (1932) Biochem. J. 26, 2129.

Battelli (1902). C.R. Soc. Biol. 54, 1518.

Bayer/

Bayer, G. and Wense, Th. (1938). Arch. Int. Pharmacodyn. 58, 103.

Beyer, K.H. (1941). J. Pharmacol. Exp. Therap. 71,151

Beyer, K.H. and Vernon,Lee W. (1942). Ibid. 74,155

Bhagvat, K., Blaschko,H. and Richter, D. (1939). Biochem. J. 33, 1338.

Bhagvat, K. and Richter, D. (1938). Ibid. 32,1397

Blaschko,H., Richter, D. and Schlossmann, H. (1937a) J. Physiol. 90,1.

Blaschko, H. et al. (1937b) Ibid. 89, 39P.

Blaschko, H. and Schlossmann,H. (1940). Ibid. 98,130.

Blix, G. (1929). Skand. Arch. Physiol. 56, 131

Burn, J.H. and Tainter, M.L. (1931). J.Physiol. 71,169

Carnot, P. and Josserand,P.(1902). C.R.So.c Biol. 54,1472.

Clark, A.J. and Raventos, J. (1939). Quart. J. exp. Physiol. 29,185.

Cramer, W. (1911). J. Physiol. 42, 36.

Curtis, F.R.(1929). J. Pharmacol. exp. Therap. 35,333

Dakin/

Dakin. (1905). Proc. Roy. Soc. B.76, 491.

De Vos,J+ Kochmann,M.(1905).Arch.Int.Pharmacodyn.14,81.

Dragstedt, C.A., Wightman, H.H. and Hoffman, J.W. (1928) Amer. J. Physiol. 84, 307

Duchateau-Bosson, G. and Florkin, M. (1939). C.R.Soc. Biol. 132, 47.

Elliott, R.T. (1905) J. Physiol. 32, 401.

Embden, G. and v.Furth,O. (1904). Beitr.Chem. Physiol. 4, 421.

Farmer, C.J. and Abt, A.F.(1936). Proc. Soc. exp. Biol. Med. 34, 146.

Frolich, A. and Loewi, O. (1910). Arch. Exp. Path. Pharmak. 62, 159.

Gaddum, J.H. and Kwiatkowski, H.(1938).J.Physiol. 94, 87.

Geoffridie. (1907). Biochem. Centr. 6, 607.

Green, D.E. and Richter, D.(1937) Biochem. J. 31,596

Heard, R.D.H. and Raper, H.S.(1933) Biochem. J. 27, 36.

Jackson, D.E.(1909). Amer. J.Physiol. 23, 226.

Jang. (1940). J. Pharmacol. exp. Therap. 70,347.

Jowett. (1904). J. Chem. 85,192.

Kastle and Loevenhart. (1901). Amer. Chem. J. 20,539

Keilin/

Keilin, D. (1929). Proc. Roy. Soc. B.104, 206

Idem. (1936). Ibid. B.121, 165.

Keilin, D. and Hartree. (1938) Proc. Roy. Soc. 125, 171.

Keilin, D. and Mann, T. (1938). Proc. Roy. Soc. B.125, 187.

Kohn, H.I. (1937). Biochem. J. 31, 1693.

Kretschmer. (1907). Arch. exp. Path. Pharmak. 57,423

Labate, J.S. (1941). J. Pharmacol. Exp. Therap. 72, 370.

Langlois. (1897). C.R. Soc. Biol. Paris, P.571.

Idem. (1898). Arch. Physiol. Norm. Path. 5 Ser. 10, 124.

Lawen,A. (1904). Arch.f.Exp.Path.u.Pharm. 51,415

Lawrence, W.S., Morton, M.C. and Tainter, M.L. (1942). J. Pharmacol. Exp. Therap. 75, 219.

Livon. (1904). C.R.Soc. Biol.P.539 + 1118

Luduena, F.P. (1940). Rev. Soc. Argent. Biol. 16,701

Macgregor, D.F.(1939). J. Pharmacol. Exp. Therap., 66, 394.

Markowitz, J. and Mann, F.C.(1929). Amer. J.Physiol. 89, 176.

Meio /

Meio de R.H. and Luduena, F.P.(1940). Rev. Soc. Argent. Biol. 16, 452.

Neuberg, C.(1908). Biochem. Z. 8, 383.

Oliver, G. and Schafer, E.A.(1895). J.Physiol. 18,230

Philpot, F.J.(1937). Biochem. J. 31, 856.

Idem. (1940). J. Physiol. 97, 301.

Philpot, F.J. and Cantoni, G.(1941). J. Pharmacol. Exp. Therap. 71, 95.

Prohaska, J.V., Haines, H.P. and Dragstdt,L.R. (1937) Ann. Surg. 106, 857.

Pugh, C.E.M. and Quastel, J.H. (1937). Biochem. J. 31, 286.

Richter, D. (1934) Biochem. J. 28, 901.
Richter, D. (1937) Ibid. 31,2022.

Idem. (1940) J.Physiol. 98, 361.

Richter, D. and Mackintosh. F.C. (1941) Amer. J.Physiol. 135,1.

Richter, D. and Tingey, A.A.(1939) J.Physiol. 97,265.

Rosenblueth, A.(1932). Amer.J.Physiol. 101,149

Idem. (1931). Ibid. 98,186.

Schild,H. (1933). J.Physiol. 79, 455

Schupfer/

Schupfer, F.(1940). Bull. Soc. Ital. Biol.Sper. 15,282.

Stolz. (1904). Ber. Chem. Ges. 37, 4149

Sugawara, T.(1928-29). Tokio J. exp. Med. 12,97

Szent Gyorgi,A. (1928). Biochem. J. 22, 1387

Tainter, M.L. (1929). J. Pharmacol. exp. Therap. 36, 569

Idem. (1930). Quart. J. Pharm. Pharmacol. 111, 584

Idem. (1931). Arch. Int. Pharmacodyn. 41, 365.

Takamine, J.(1901). Amer.J.Pharmacy. 73,523.

Tatum, A.L.(1912). J.Pharmacol. Exp. Therap. 4,151

Idem. (1920). Ibid. 16, 109.

Thienes, C.H. and Hockett, A.J. (1928). Proc. Soc. Exp. Biol. 25, 793.

Toscano-Rico, J., Malafaya, Baptista, H. (1935). C.R.Soc. Biol. Paris, 120, 545.

Trendelenburg, P.(1916). Arch. exp. Path. Pharmakol. 79, 154.

Idem. (1924). Heffter's Handb. exp. Pharmak. 2, 1192.

Tripod/

Tripod, J. (1940). J.Physiol. 97, 289.

Vulpian. (1856). C.R. 43,663.

Welch, A.de M. (1934). Amer. J.Physiol. 108, 360.

Weinstein, S.S. and Manning, R.I. (1937). Sci. 86,19

Wense, Th. (1935). Hoppe Seyl.Z. 260,100.

Wiltshire, M°P. (1931). J.Physiol. 72, 88.

Wirt, S.K. and Tainter, M.L.(1932). J.Pharmacol. Exp. Therap. 44, no.3

APPENDIX.

Table 1.

The effect of quick and continuous adrenaline injections on the nictitating membrane and blood pressure of cat (6 experiments).

	Quick adrenaline μdose μg/kg.	Response n.m. in mm.	Rise of B.P. in mm.	Continuous adrenaline μg/kg./min.	Height of plateau response in mm.	
					n.m.	B.P.
(1)	0.28	0.5	6	3.84	79	75
	0.84	1.5	12	5.07	80.5	62
	2.8	15	34			
	8.4	41	60			
	28.0	64,70	90,78			
	58.0	80.5	-			
(2)	0.31	1	-	0.416	1	10
	0.93	1.5	12	1.38	21	
	3.1	14	22			
	9.3	38	52			
	31.0	57	71			
(3)	0.28	no response	5	0.34	-	13
	0.84	1	12	1.13	3	30
	2.8	9	28			
	8.4	36	54			
	28.0	75	79			
(4)	0.38	0.5	11.5	0.51	0.5	28
	1.15	1	30	1.7	25	56
	3.8	17	60	5.1	55	-
	11.5	42	74			
	38.0	67	85			
(5)	0.35	0.5	6	0.466	2.5	12
	1.05	2.75	11	1.55	32	36
	3.5	22	34	4.66	63	64
	10.5	43	58			
	35.0	64	82			
(6)	0.27	0.5	6			
	0.81	1.5	-	0.36	1	-
	2.7	11.5	11.5	1.088	24	-
	8.1	36	35			
	27.0	65	57			

Table 2.

Response of nictitating membrane to quick injection and continuous infusion of adrenaline in 4 sensitive (A) and 2 insensitive (B) cats.

	Quick adrenaline dose µg/kg.	Applitude of n.m. response in mm.	Continuous adrenaline dose µg/kg/min.	Plateau response of n.m.
	(A)			
(1)	0.49	1,1	0.49	0.5
	0.98	2	0.98	2
	1.47	3	1.47	9
	2.94	6	2.9	65
	4.9	19	9.8	128
	14.7	85		
	49.0	108		
	147.0	131		
(2)	0.33	1	0.9	13
	1.0	3	1.8	64
	3.3	42.5	3.42	106
	6.6	64		
	10.0	75.5		
	33.3	120		
	100.0	132		
(3)	0.4	1.75	1.21	36
	1.4	8.5	3.8	93
	4.8	43	3.13	100
	14.4	56		
	48.0	114		
(4)	0.38	1	1.27	7
	1.014	2.5		
	3.38	11		
	10.14	41		
	33.8	78		
	(B)			
(1)	0.3	no response	1.8	10
	0.72	"	3.6	20
	0.36	"		
	3.6	7,9		
(2)	0.34	no response	0.34	no response
	1.02	1.5	1.02	2
	3.4	7,5	3.4	57
	10.2	40	6.8	69
	34.0	70	10.2	88
	68.0	86		
	102.0	100		

Table 3.

Efeect of adrenaline on the nictitating membrane and blood pressure given as quick as possible and spread over different times (4 experiments)

Adrenaline dose µg/kg.	Height of response in mm. to adrenaline dose given as quick as possible and spread over different times. Nictitating Membrane					Blood Pressure				
	Quick	3 sec.	10 sec.	60 sec.	120 sec.	Quick	3 sec.	10 sec.	60 sec.	120 sec.
0.4	10.5	10	-	-	-	23	23	-	-	-
4.0	34	33	-	17	-	57	-	-	27	-
0.33	33	-	17.5	6.5	5	30	30	30	30	29
0.66	165	-	17	-	-	-	-	-	-	-
3.3	42	-	40	48	39.5	48	-	49	60	-
0.36	-	-	-	-	-	10	10	-	-	-
3.6	8	9	9	5	-	13	20	19	20	-
0.8	11	-	-	9	-	24	-	-	-	22

Table 4.

Continuous adrenaline doses and quick equivalents from blood pressure and nictitating membrane response (averages from 27 experiments).

Continuous adrenaline µg/kg./min.	Quick adrenaline equivalent µg/kg.	
	Nictitating membrane	Blood pressure
0.35	0.58	0.91
1.23	3.31	4.3
1.87	8.7	5.5
3.34	11.7	-
3.87	-	10.92
4.8	33.3	-
8.3	74.76	

Table 5.

Continuous adrenaline doses and quick equivalents from blood pressure and nictitating membrane response. (Averages from 16 experiments)

Continuous adrenaline µg./kg./min	Quick adrenaline equivalent (µg./kg.)	
	Nictitating membrane	Blood pressure
0.316	0.4	0.79
0.46	0.83	2.0
1.0	2.8	3.9
3.16	14.13	15.87
8.91	70.72	-
10.0	79.3	-

Table 6.

Duration of response of nictitating membrane to quick adrenaline injections. (Averages from 8 experiments)

Dose adrenaline µg./kg.	Duration of nictitating membrane response in min.	No. of observations
0.38	2.5	7
1.15	3.59	8
3.77	5.93	8
11.48	8.5	8
37.8	14.7	8

Table 7.

Duration of response of nictitating membrane to quick adrenaline injections. (Averages of 8 experiments)

Dose of adrenaline µg/kg.	Duration of nictitating membrane response in min.	No. of observation
0.45	4.58	6
1.19	5.0	8
4.26	8.44	9
12.93	12.75	6
46.67	20.5	7

Table 8

Adrenaline dosage.							
(a) continuous µg/kg./min.	0.34	0.34	1.02	3.4	8.0	6.8	10.2
(b) additional quick injection µg/kg.	1.0	3.4	3.4	10.2	10.2	10.2	10.2
Response n.m. after (a)	0.5	0.5	2	17	57	69	88
(b)	2	7.5	12	51	83	91	101
Adrenaline equivalents of (a)	0.5	0.5	1.2	3.8	19	28	60
(b)	1.2	2.2	3.0	14	50	70	110
Difference	0.7	1.7	1.8	10.2	31	42	50

Table 9.

Continuous dose g/kg./min.	Additional dose g/kg.	Value of additional dose g/kg. calculated from response.
0.33	1.02 3.4	1.5,1.0 1.7
0.5	1.5	0.4, 2.5
0.53	5.0 11.5	5.0 11.7
0.66	3.3	2.3
1.0	3.4 5	3.53, 1.0 7
1.5	15	18
1.66	6.6	8.7
1.79	11.5	12.92
2.66	10	16
3.4	10.2	10
4.0	10	15
5.0	10 15	29 100
5.3	11.5	21.9
6.8	10	45
10	10 15	40 10
15	49	64

Table 10.

Amplitude of the response of nictitating membrane to quick and continuous injections before and after pyrogallol administration (4 experiments).

Expt.	Dose of adrenaline µg/Kg	N.m.response before pyrogallol mm.	N.m.response after pyrogallol mm.	Continuous adrenaline µg/Kg/min	Ht.of plateau attained before pyrogallol	Ht.of plateau attained after pyrogallol
1	0.32	1	-	0.346	1	23.5
	0.96	4	9	3.46	69.5	98.5
	3.2	31	-			
	9.6	63	83			
	32.0	82	-			
2	0.29	1.5	-	0.35	3	5
	0.88	4	3.5	3.5	49	110
	2.93	18				
	8.8	45	92			
	29.3	82				
3	0.23	1	-	0.26	1.5	11.5
	0.69	2	2.5	2.65	47	117
	2.3	20	-			
	6.9	55	106			
	23.0	90	-			
4	0.33	0.5	2.5	0.14	0.5	3
	0.99	1.5	12	0.44	2.5	22
	3.3	22.5	64	1.48	55	91.5
	9.9	59	107			
	33.3	94	115			

Table 11.

Continuous adrenaline doses and quick equivalents before and after pyrogallol.(averages of 8 experiments)

Continuous adrenaline µg/kg./min.	0.16	0.26	0.4	2.04	4.4	5.0
Quick adrenaline equivalent µg/kg. before pyrogallol	-	0.35	0.59	5.7	15.8	21
Quick adrenaline equivalent µg/kg. after pyrogallol	0.48	-	1.79	10.2	62.8	-

Table 12.

Duration of nictitating membrane response to quick adrenaline doses before and after pyrogallol from the averages of the number of observations given in bracket.

Before pyrogallol		After pyrogallol	
Dose of adrenaline µg/kg.	Duration of response in min.	Dose of adrenaline µg/kg.	Duration of response in min.
0.31 (4)	2.7	0.3 (3)	3.5
0.9 (7)	3.1	0.86 (6)	5.95
3.0 (7)	5.46	2.64 (6)	9.52
9.03 (7)	8.5	9.1 (6)	20.25
30.08 (7)	14.1	31.82 (4)	32.5

Table 13.

The effect of intravenous cocaine on the sensitivity to adrenaline (from 19 experiments)

Intravenous dose of cocaine mg/kg.	No. of cats	No. of cats sensitized to doses of adrenaline up to 50 µg/kg.	No. of cats sensitized to doses of adrenaline under 15 µg/kg. only
1.5 -2.5	13	11	2
3.0 -5.0	3	2	1
5.0-10.0	3	nil	3

Table 14.

Amplitude of the response of nictitating membrane to quick and continuous adrenaline injection before and after cocaine administration (3 experiments).

	Dose adrenaline µg/kg.	N.m.response before cocaine to n.m.	N.m.response after cocaine in mm.	Continuous adrenaline dose µg/kg./min.	Ht. of plateau attained before cocaine	Ht.of plateau attained after cocaine
1	0.39	1.5	21.5	0.52	11	70
	1.17	10	34	1.74	61	85
	3.92	38	-	0.174	-	42
	11.76	70	-			
	39.2	95	95			
2	0.303	0.5	18	0.404	2.5	36
	0.909	1.5	28.5	1.35	49	34
	3.03	25.5	58.5	0.04	-	5
	9.09	59.5	80	0.135	-	23
	30.3	78	89			
	0.03	-	4			
	0.09	-	3			
3	0.44	0.5,1	20	0.195	1	36
	1.32	12.5	-	0.585	17	53
	4.4	43	-	1.95	62	86.5
	13.2	70	71	0.058	-	12.5
	44.4	91	-	0.019	-	5

Table 15.

Response of the blood pressure to quick and continuous adrenaline injections before and after cocaine administration (7 expts)

No: Exp.	Dose adrenaline µg/kg. quick	Response blood pressure to quick adrenaline in mm.		Dose adrenaline µg/kg/min. continuous	Plateau response of blood pressure to continuous adrenaline in mm.	
		Before cocaine	After cocaine		Before cocaine	After cocaine
1	0.31		11.5	0.416	10	33
	0.93	12	22	0.138	-	16
	3.1	22	44	1.38	-	51
	9.3	52	-			
	31.0	71	-			
2	0.39	9	8.5	0.174	-	17
	1.17	-	9.5	0.5	12	27
	3.9	19		1.74	30	39
	11.7	37				
	39.2	63				
3	0.35	6	13	0.46	11	-
	1.05	11	20	4.6	64	-
	3.5	34	22			
	10.5	58	-			
	35.0	82	-			
4	0.04	5	5			
	0.12	6	-			
	0.4	10	7			
	1.2	15				
	4.08	40	26			
	12.2	55	60			
	40.8	69	79			
5	0.028	-	6.5	0.038	-	4
	0.089	-	7.5	0.114	-	17
	0.289	7	13,6.5	0.38	-	38
	0.86	7	20	1.14	-	52
	2.189	30	38			
	8.67	59	54			
	28.9	69	81			
6	0.317	6		4.34	60	61
	1.05	9				
	3.17	27				
	10.5	59				
	31.7	85				
	63.4	-	89			
7	0.312	7	10	1.35	-	47
	0.936	14	22	4.07	76	83
	3.12	36	39			
	9.36	60	38			
	31.2	80	63			

Table 16

Continuous adrenaline and quick equivalents before and after cocaine administration. (Averages of 5 experiments with nictitating membrane response)

Continuous adrenaline µg/kg./min.	0.2	0.53	1.8	3.6	5.0
Equivalent of quick dose µg/kg. before cocaine		0.66	4.4	13.1	28.1
Equivalent quick dose µg/kg./after cocaine	0.44	2.8	17.7	-	-

Table 17

Duration of nictitating membrane response to adrenaline doses before and after cocaine from the averages of the number of observations given in the bracket.

Before cocaine		After cocaine	
Dose adrenaline µg/kg.	Duration of response in min.	Dose adrenaline µg/kg.	Duration of response in min.
0.38 (6)	2.29	0.3 (2)	5.25
1.1 (10)	3.27	0.75 (7)	6.72
3.65 (9)	5.47	1.24 (4)	10.12
11.24 (8)	7.9	3.43 (4)	13.75
35.7 (7)	15.1	10.9 (5)	22.4
		45.5 (2)	32.5

Table 18.

	Ascorbic acid in n.m.		Amount of ascorbic acid given mg./kg. body wt.	Method of administration of ascorbic acid
	Before administration of ascorbic acid mg.%	After administration of ascorbic acid mg. %		
1.	0.011,0.01		none	
2.	0.015, 0.016		none	
3.	0.016	0.036	320	Continuous intravenous
4.	0.06	0.05	327	do.
5.	0.04	0.08	637	do.
6.	0.009	0.12	645	do.
7.	0.011	0.15	620	do.
8.	0.011	0.15	673	Single, intravenous
9.	0.011	0.1	563	do.

Note: In experiments 1 and 2 adrenaline was injected between the removal of the n.m. This had obviously no effect on the ascorbic acid content.

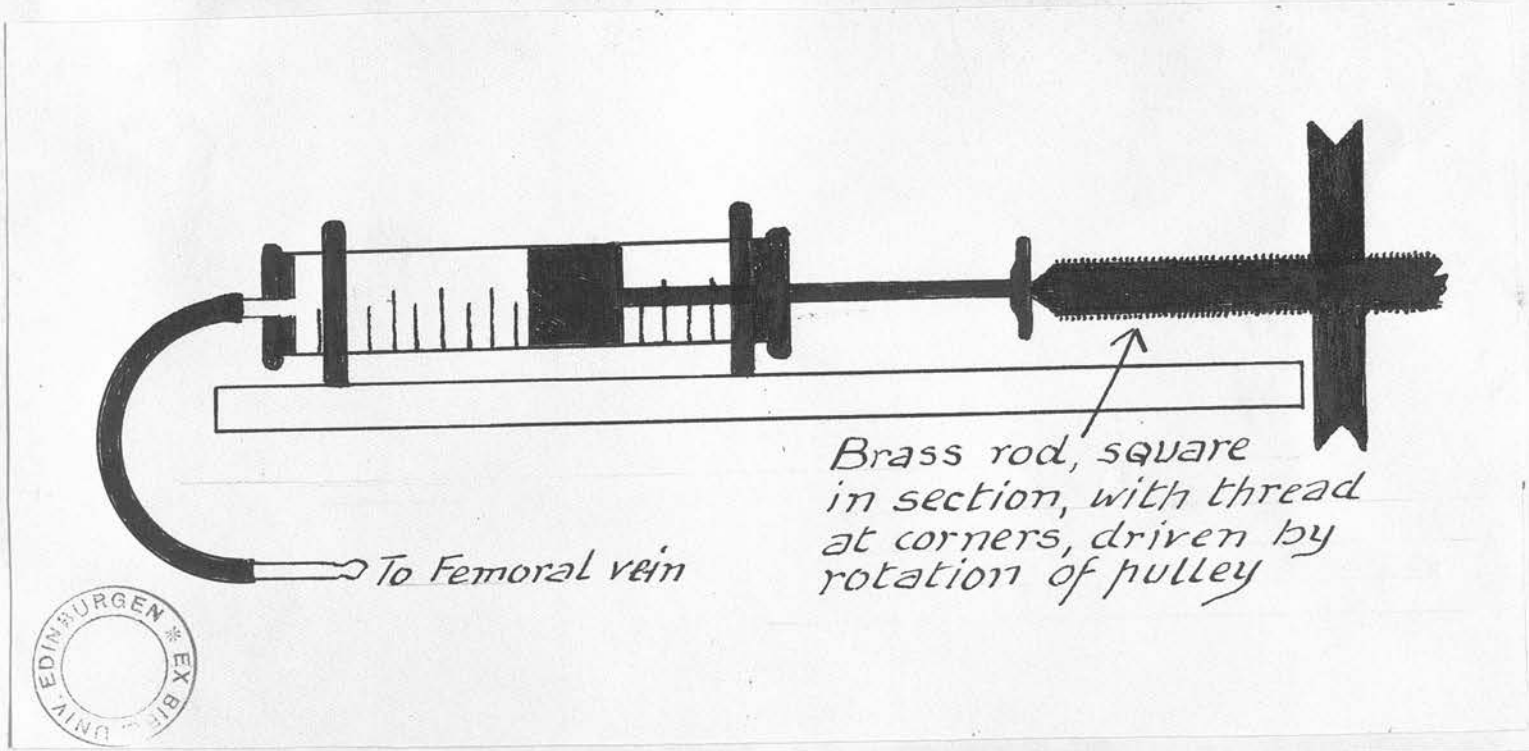


Fig. 1. Diagrammatic sketch of continuous infusion apparatus (details given in the text).

Fig. 2a. Responses of the n.m. and blood pressure to quick adrenaline doses.

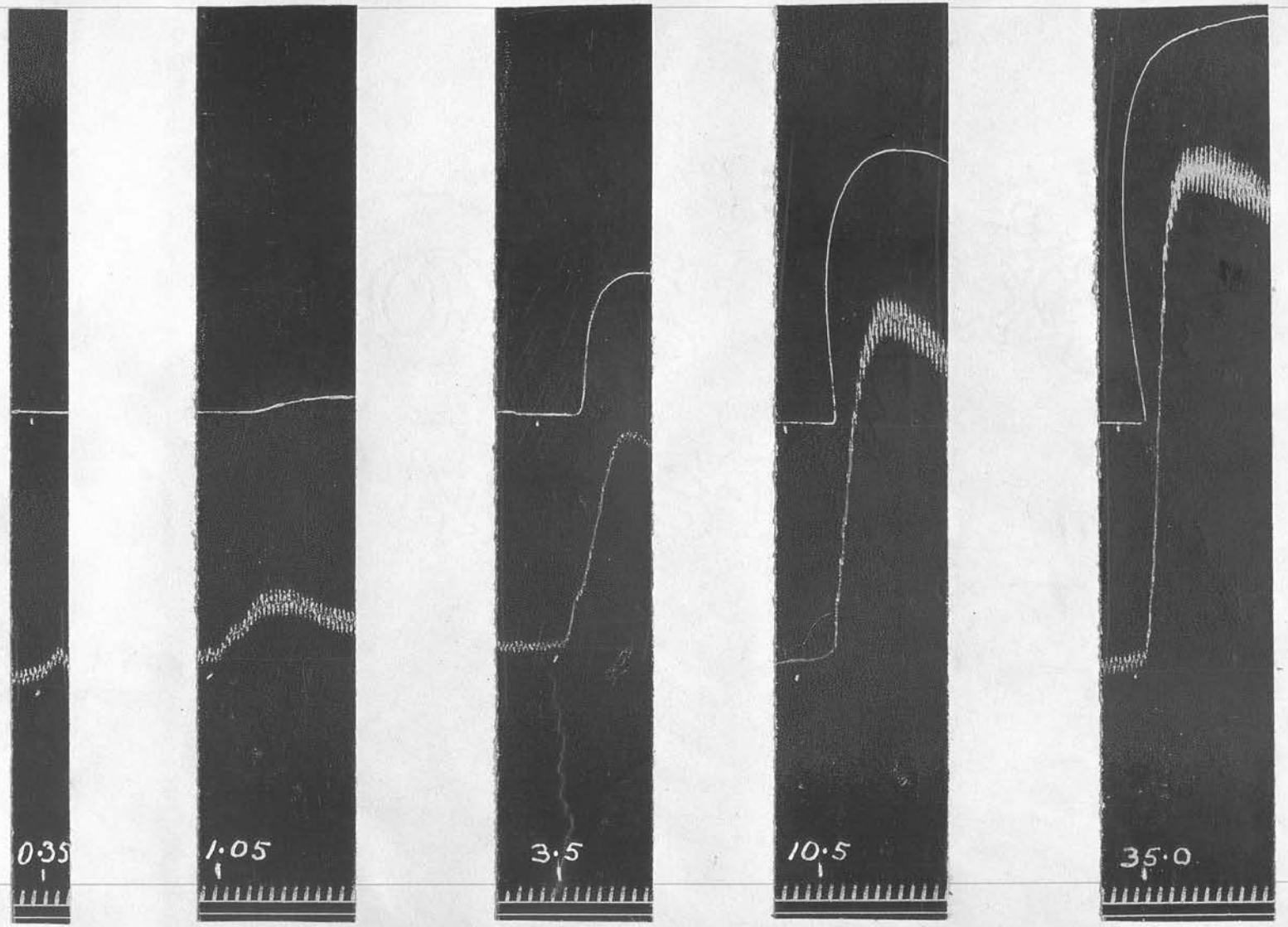


FIG. 2a.

Fig. 2b. Responses of the n.m. and blood pressure to continuous infusion of 0·4664 g/kg/min. adrenaline. The infusion stopped at "S."

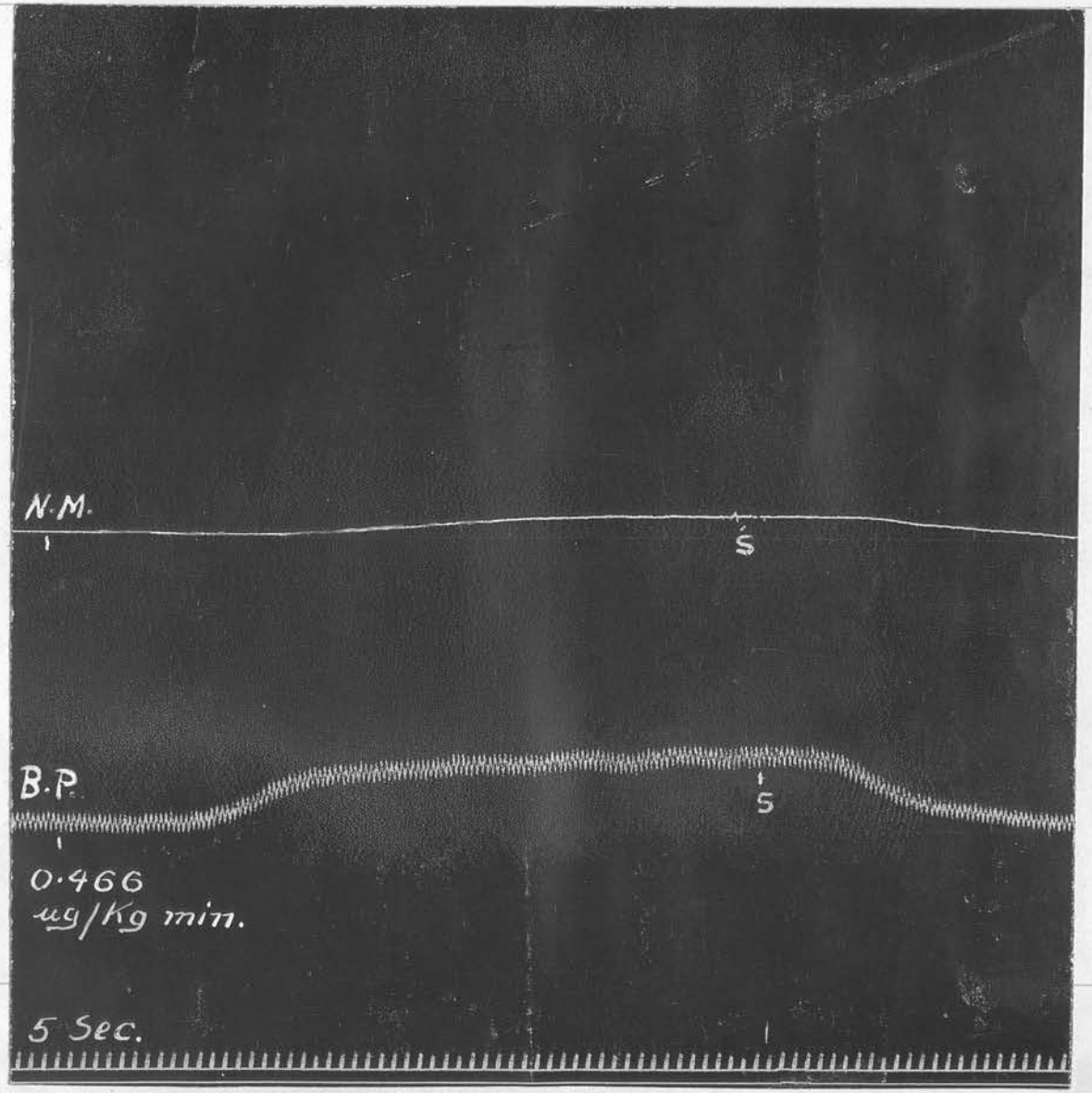


FIG.2b.

Fig. 2c. Responses of the n.m. and blood pressure to continuous adrenaline infusion of 1.55 µg/kg/min. The infusion was stopped at S.

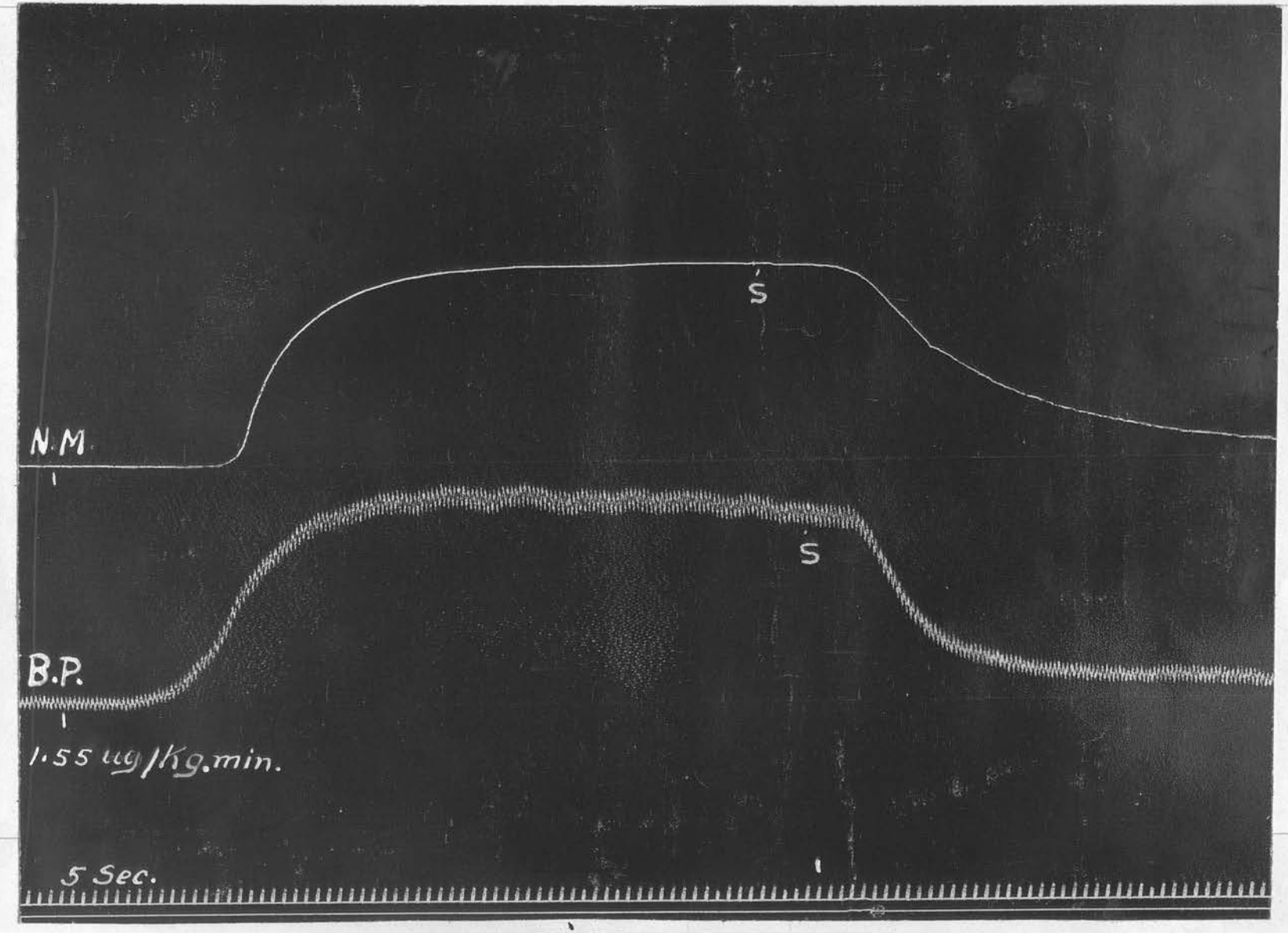


FIG.2 c.

Fig. 2d. Responses of the n.m. and blood pressure to continuous adrenaline infusion of 4.66 μg/kg/min. The infusion was stopped at S.

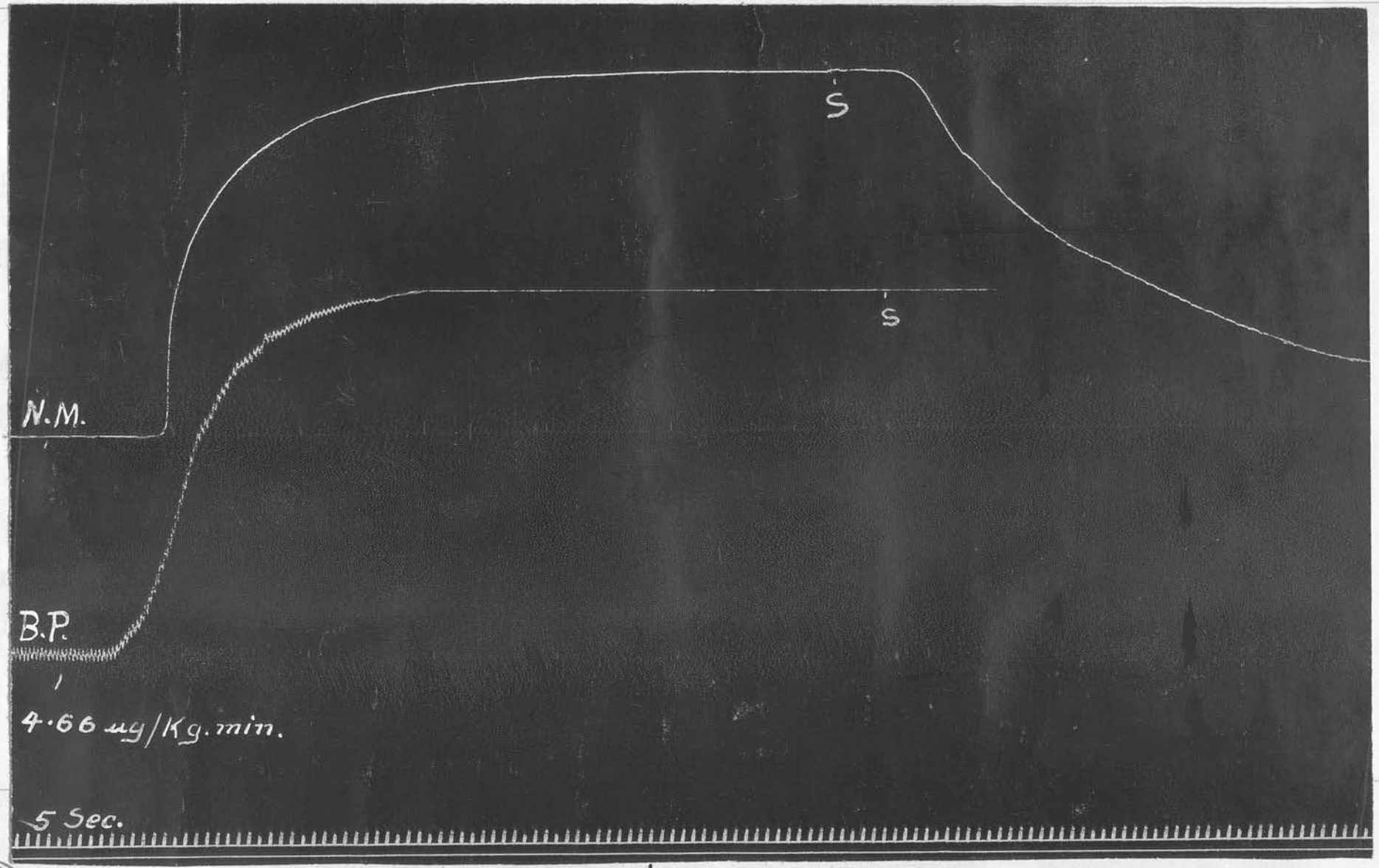


FIG.2d.

Fig. 3a. Responses of the n.m. and blood pressure to quick adrenaline doses.

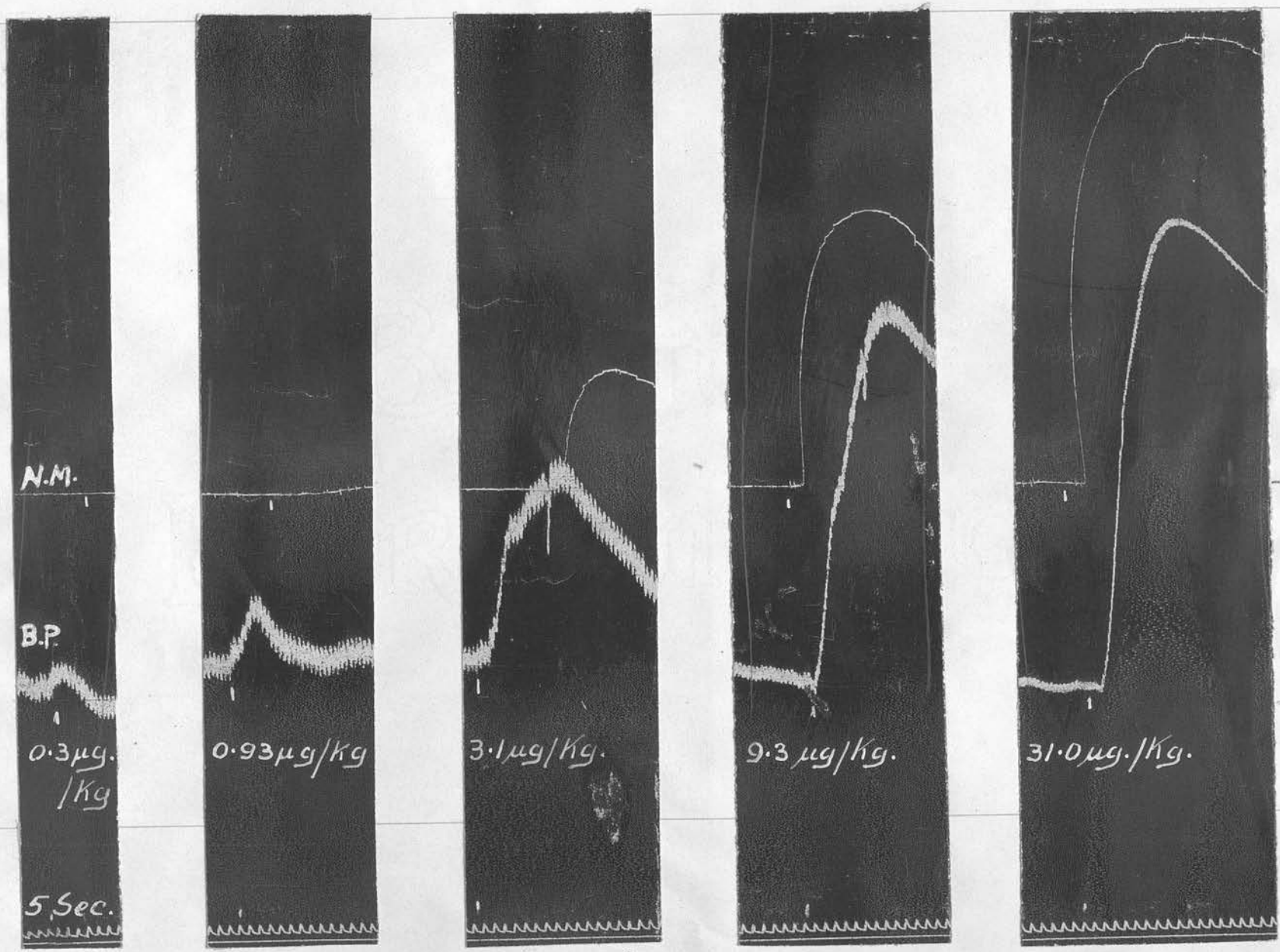


FIG.3a.

Fig. 3b. Responses of the blood pressure and n.m. to continuous adrenaline infusion of 4.07 µg/kg/min. The infusion was stopped at S.

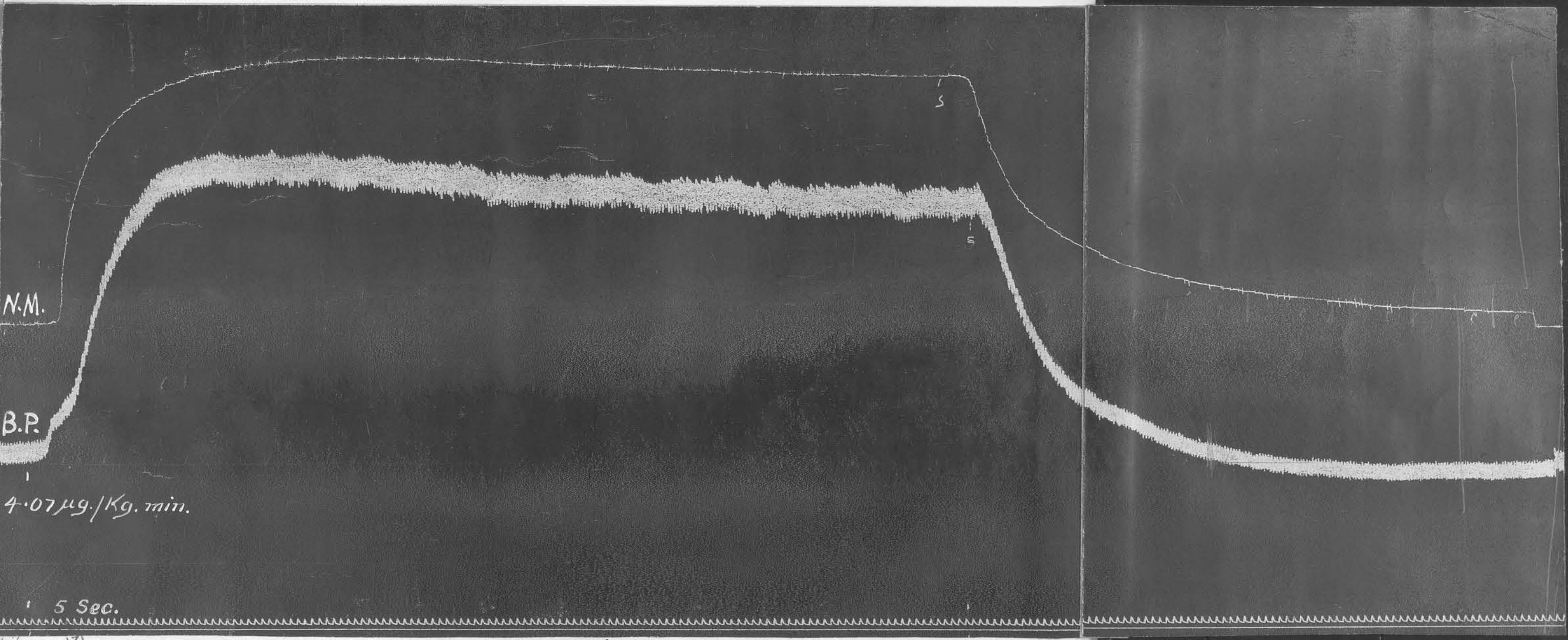


FIG.3b.

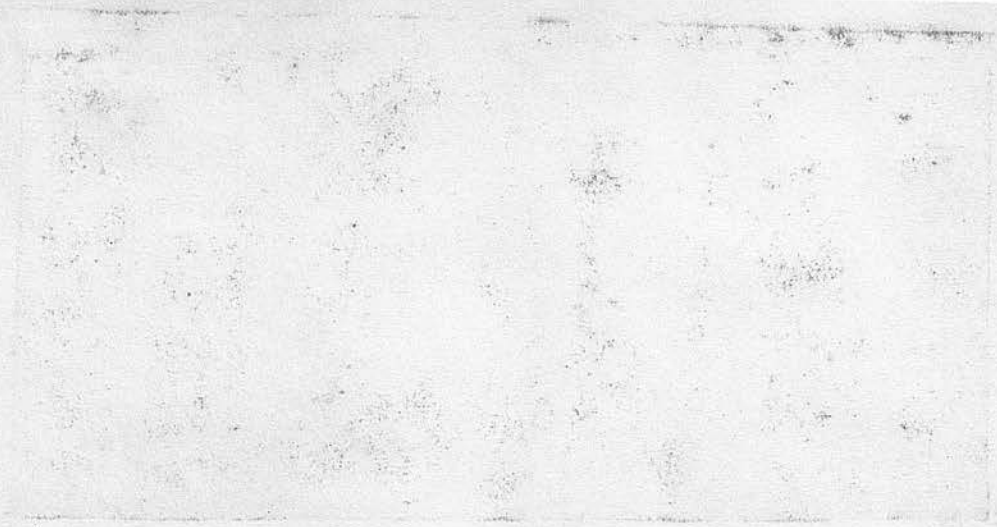


Fig. 4a. Response of the n.m. to various quick adrenaline doses.

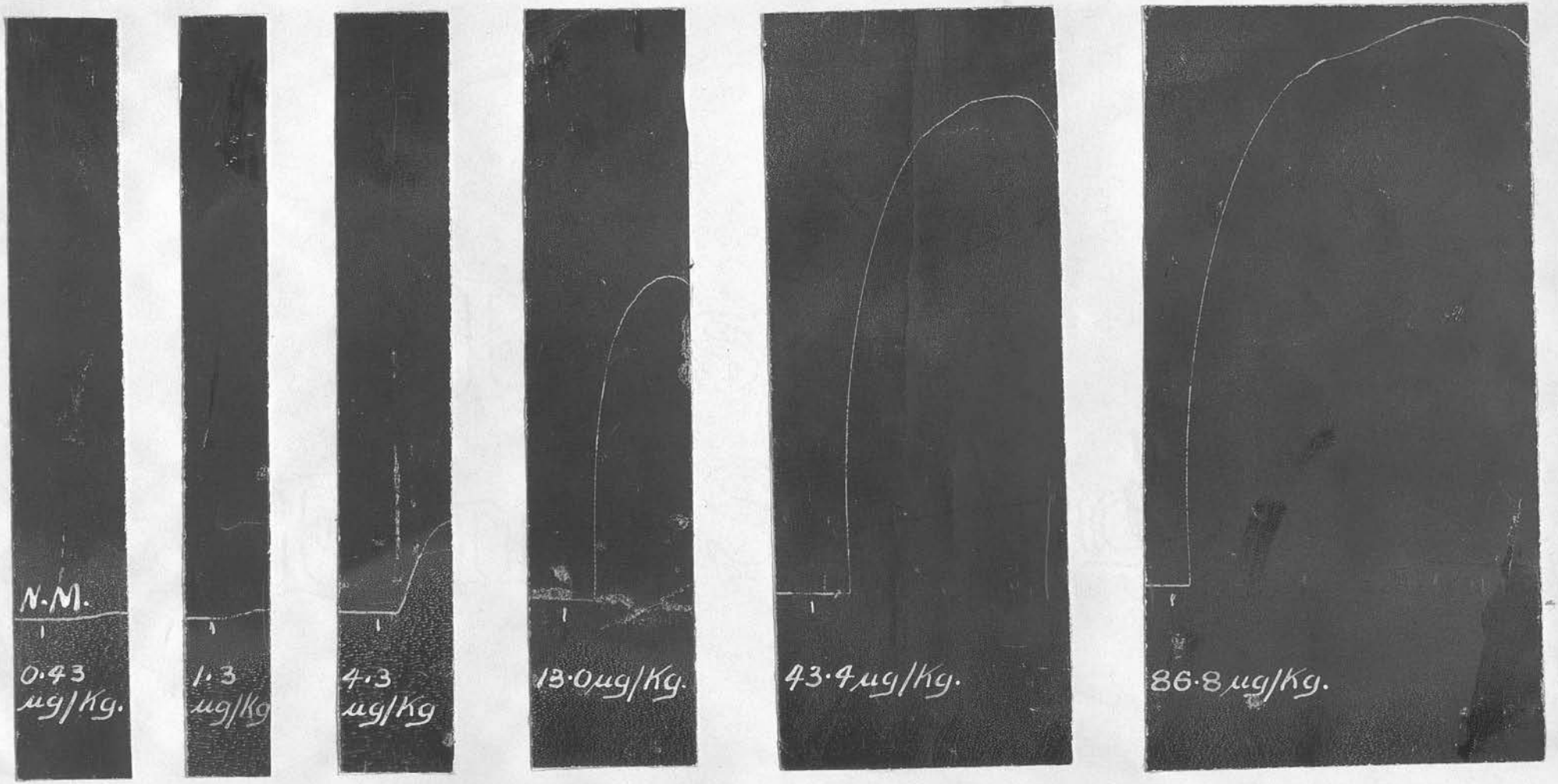


FIG.4a.

Fig. 4b. Plateau response of the n.m. to continuous dose of 6.02 μg/kg/min. adrenaline administered for 19 mins. The gap in the figure shows an interval of 12 mins. The infusion was stopped at S.

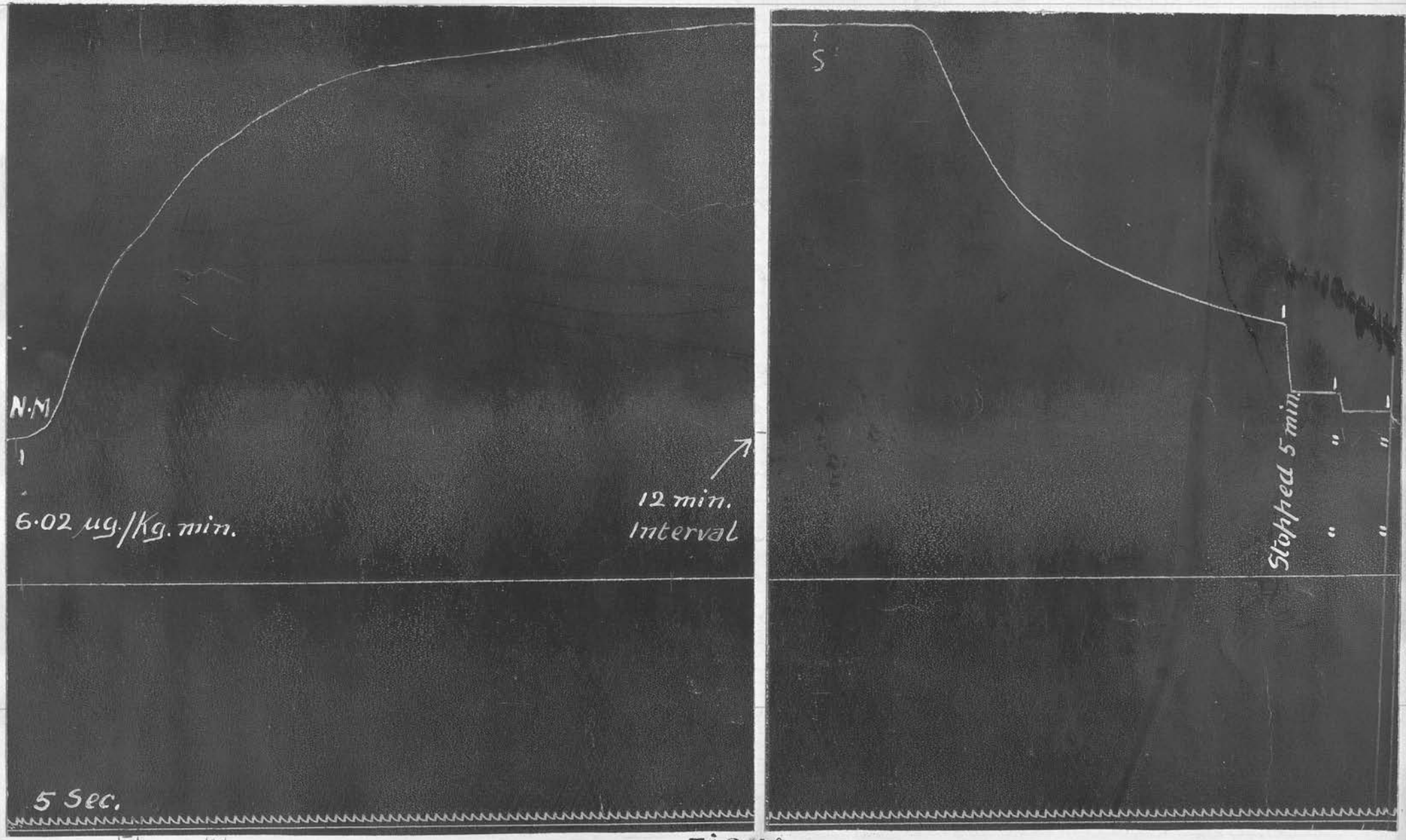


FIG. 4b.

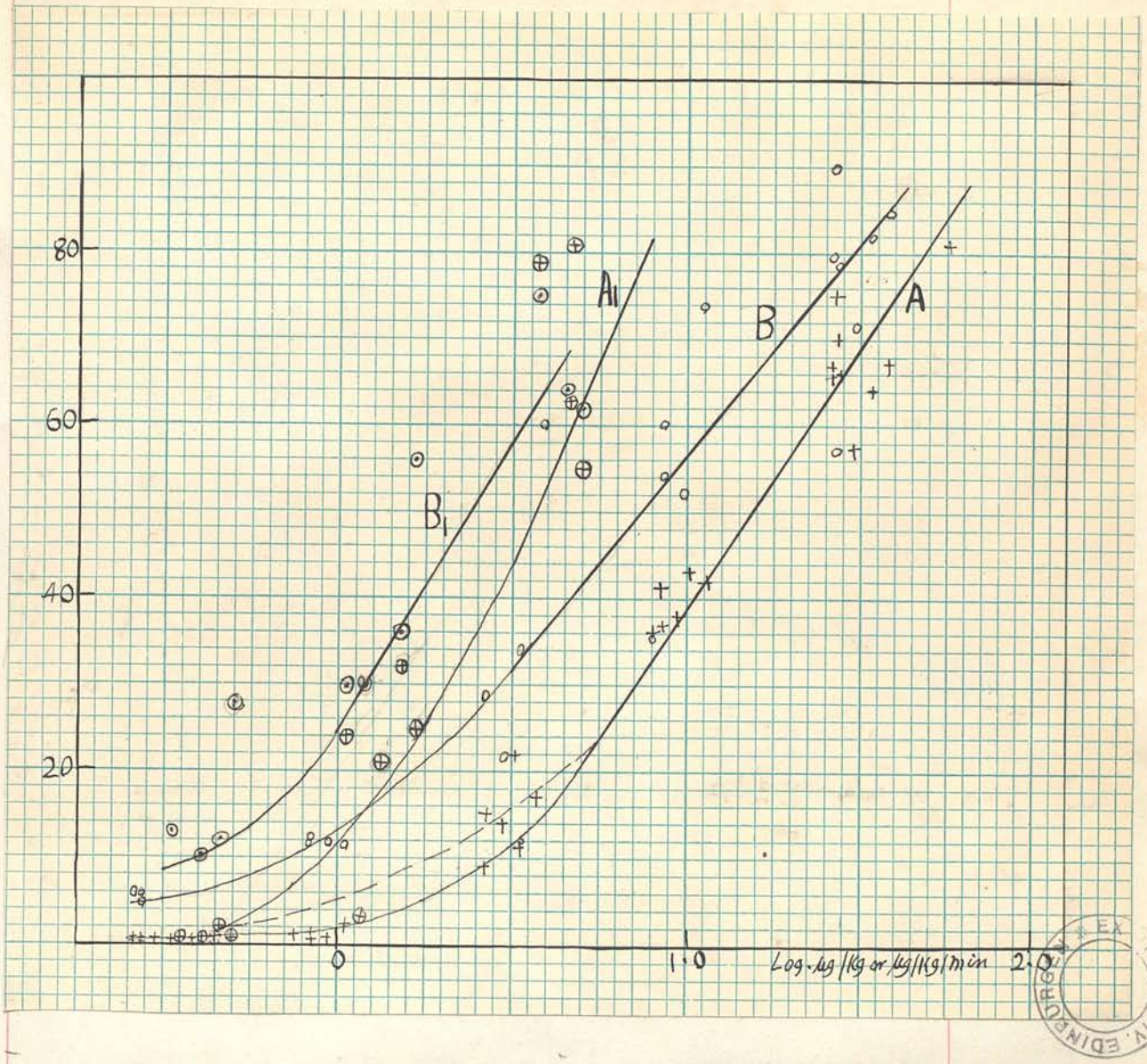


Fig. 5. Effects produced on the nictitating membrane and blood pressure of cat by quick (A and B) and continuous (A_1 and B_1) doses of adrenaline. Figures from 6 experiments given in Table 1.

Abscissa: log dose µg/kg. or µg/kg./min. adrenaline
Ordinate: height of response of nictitating membrane or blood pressure.
The dotted line shows where the curve drawn to the formula x 1/k = y/100-y, deviates from the observed value. The value of maximum response both for blood pressure and n.m. = 100 mm. and k = 15 and 7 µg/kg. for curves A and B respectively.

Fig. 6. Effects produced by quick (A and B) and continuous (A_1 and B_1) doses of adrenaline. A and A_1 figures from 4 sensitive cats (Table 2a) and B and B_1 figures from 2 insensitive cats (Table 2b).

Abscissa: log. dose µg/kg. or µg/Kg/min.

Ordinate: height of response of n.m. in mm.

The dotted line shows where the curves drawn to the formula $x_0 \; 1/k = y/100-y$ deviate from the observed values.

Maximum response = 150 mm. and values of k = 10 and 31 µg/kg.

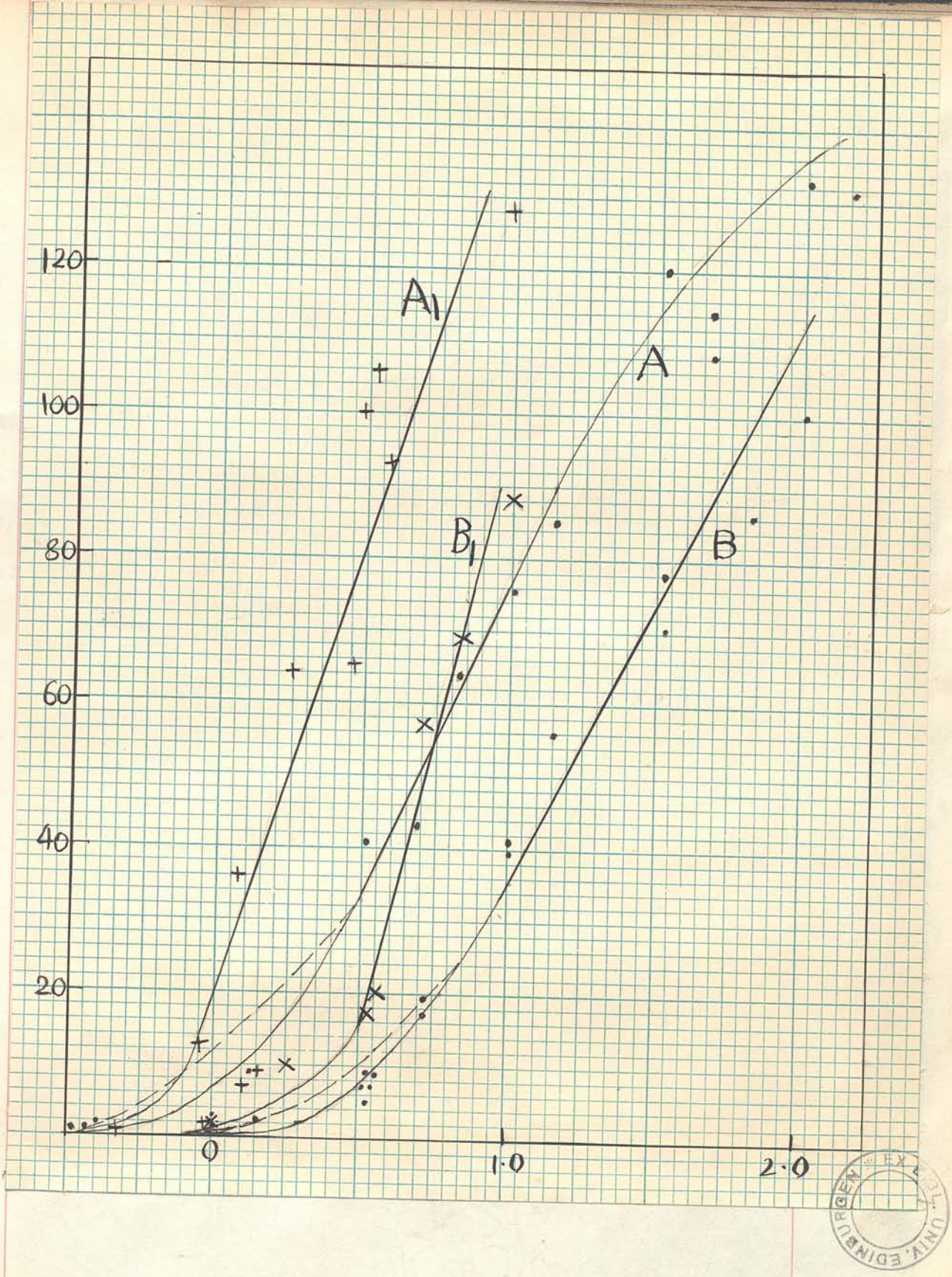


Fig. 6.

Fig. 7. Response of the n.m. and blood pressure to continuous dose of 10.1 µg/kg/min. adrenaline. At "P" quick adrenaline dose (12.6 µg/kg/min.) was administered which produced an extra response.

Blood pressure shows a lowering of the level during the period of infusion. At S, the infusion was stopped.

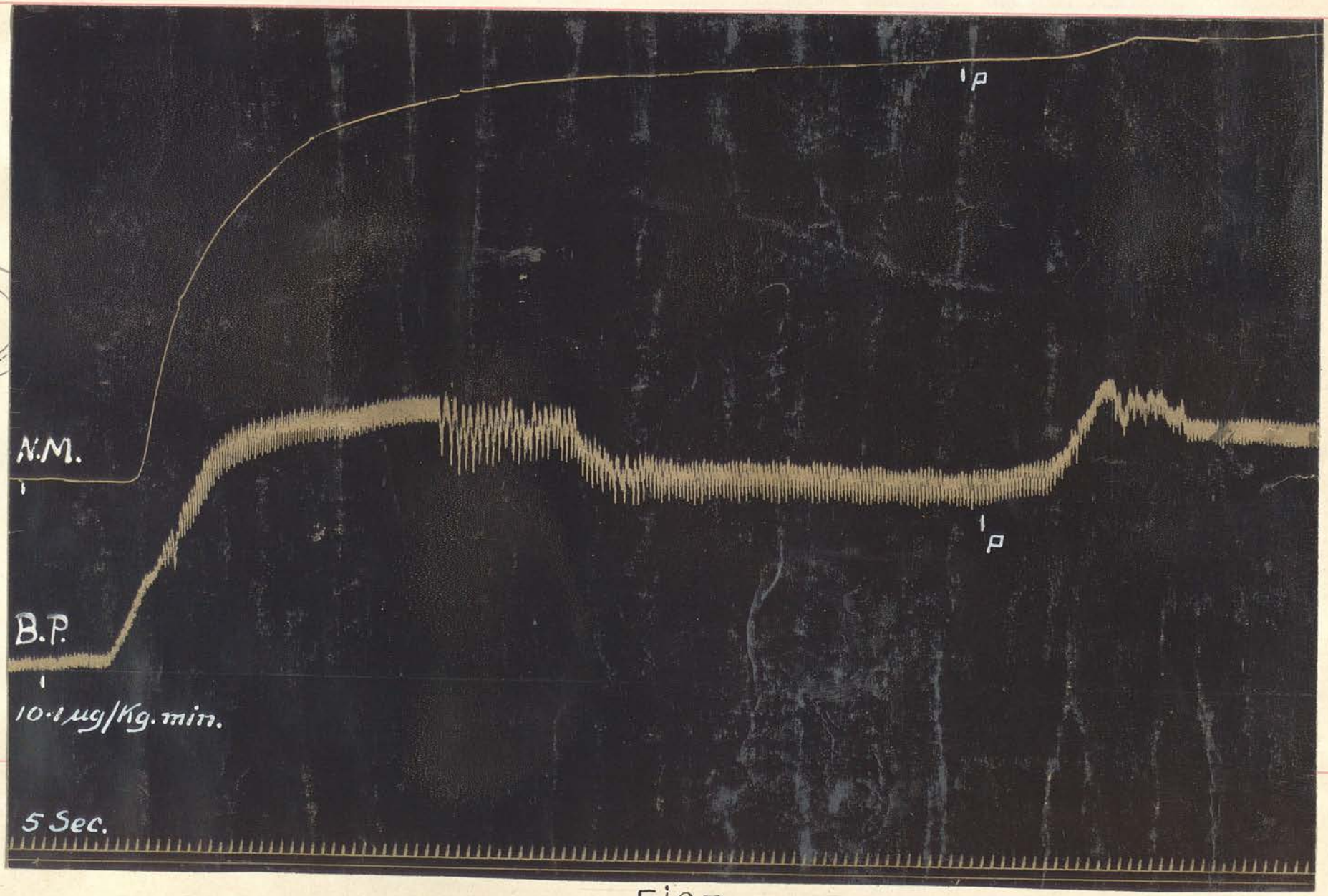


FIG.7.

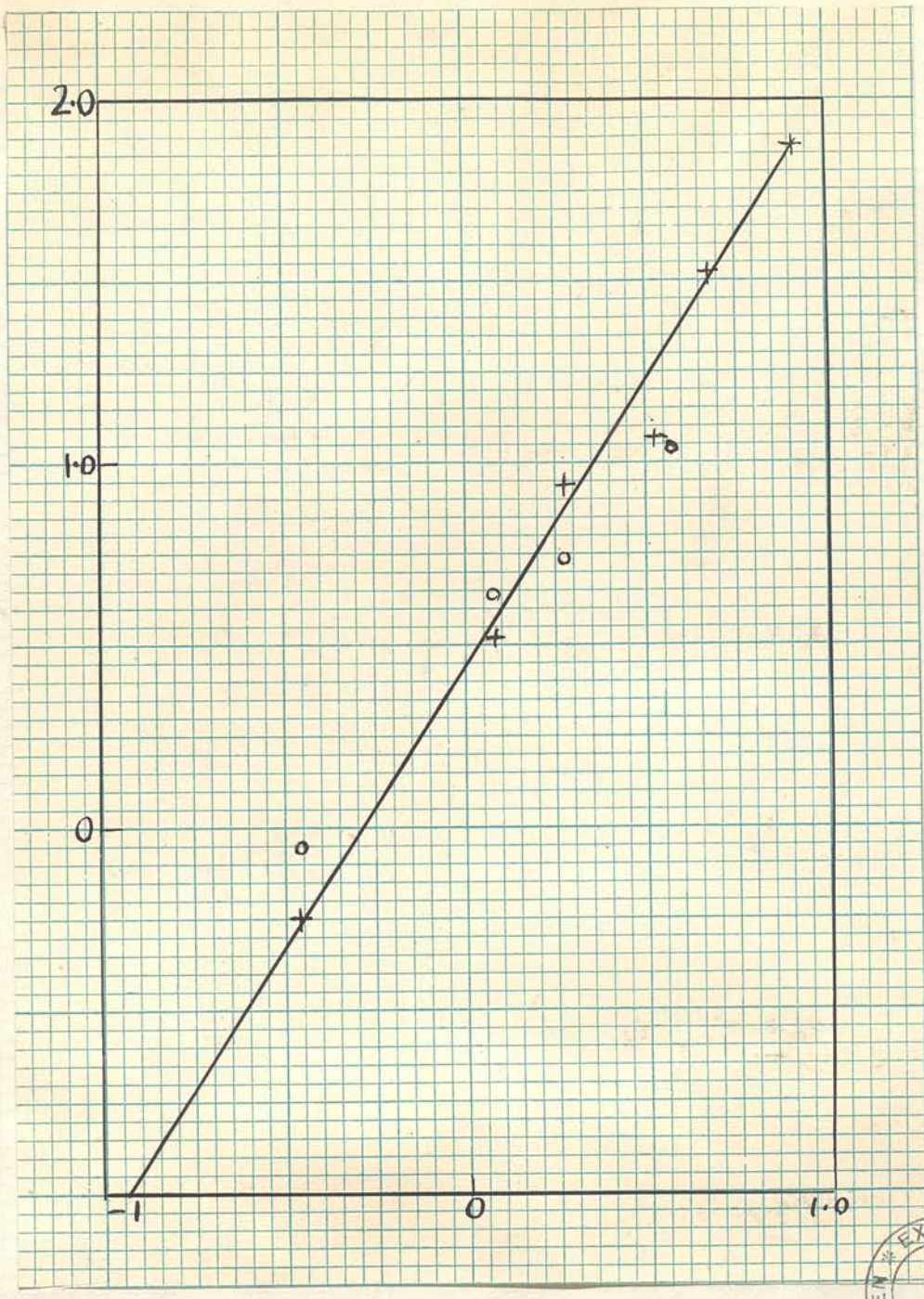


Fig. 8. Relation between log continuous dose µg/kg./min. (abscissa) and log quick dose µg/kg. (ordinate) of adrenaline which produce equal effects. Averages from 27 experiments (Table 4)
Crosses: nictitating membrane.
Circles: blood pressure.

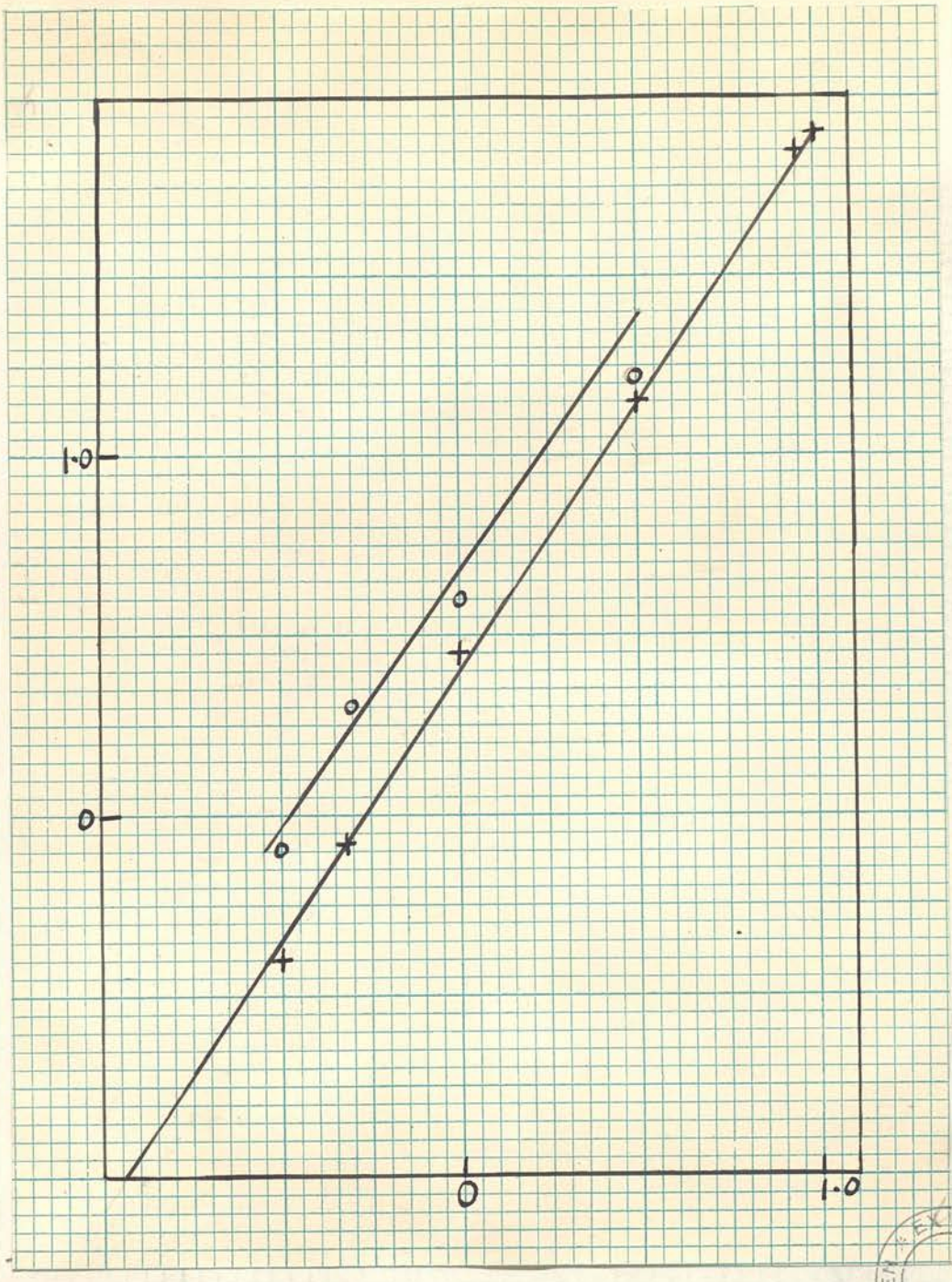


Fig. 9. Relation between log. continuous dose µg/kg./min. (abscissa) and log. quick dose µg/kg. (ordinate) of adrenaline which produce equal effects. Averages from 16 experiments (Table 5)

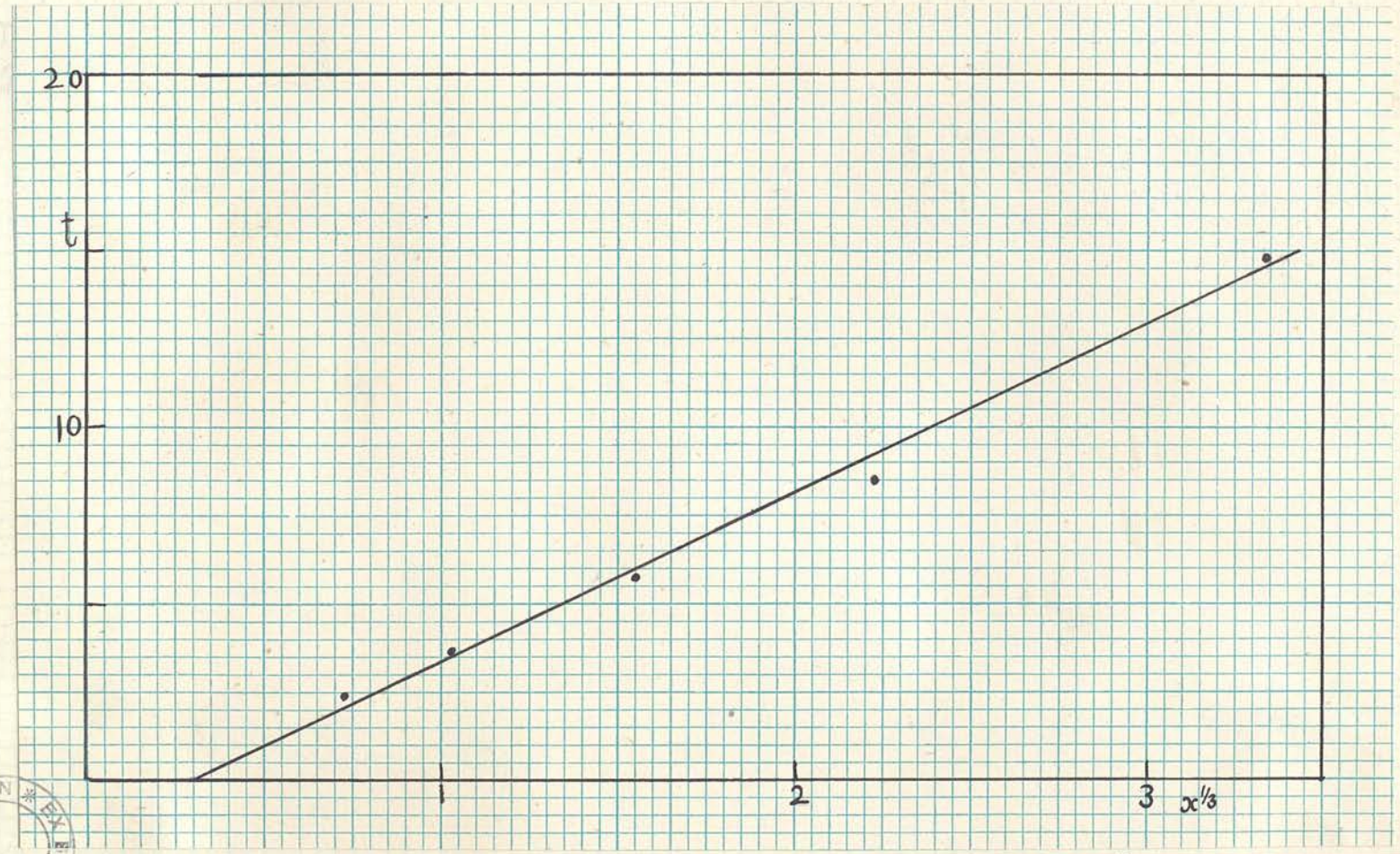


Fig. 10. Relation between adrenaline (dose µg/kg.)$^{1/3}$ (abscissa) and duration of nictitating membrane response in min. (ordinate). Averages of 8 experiments (Table 6)

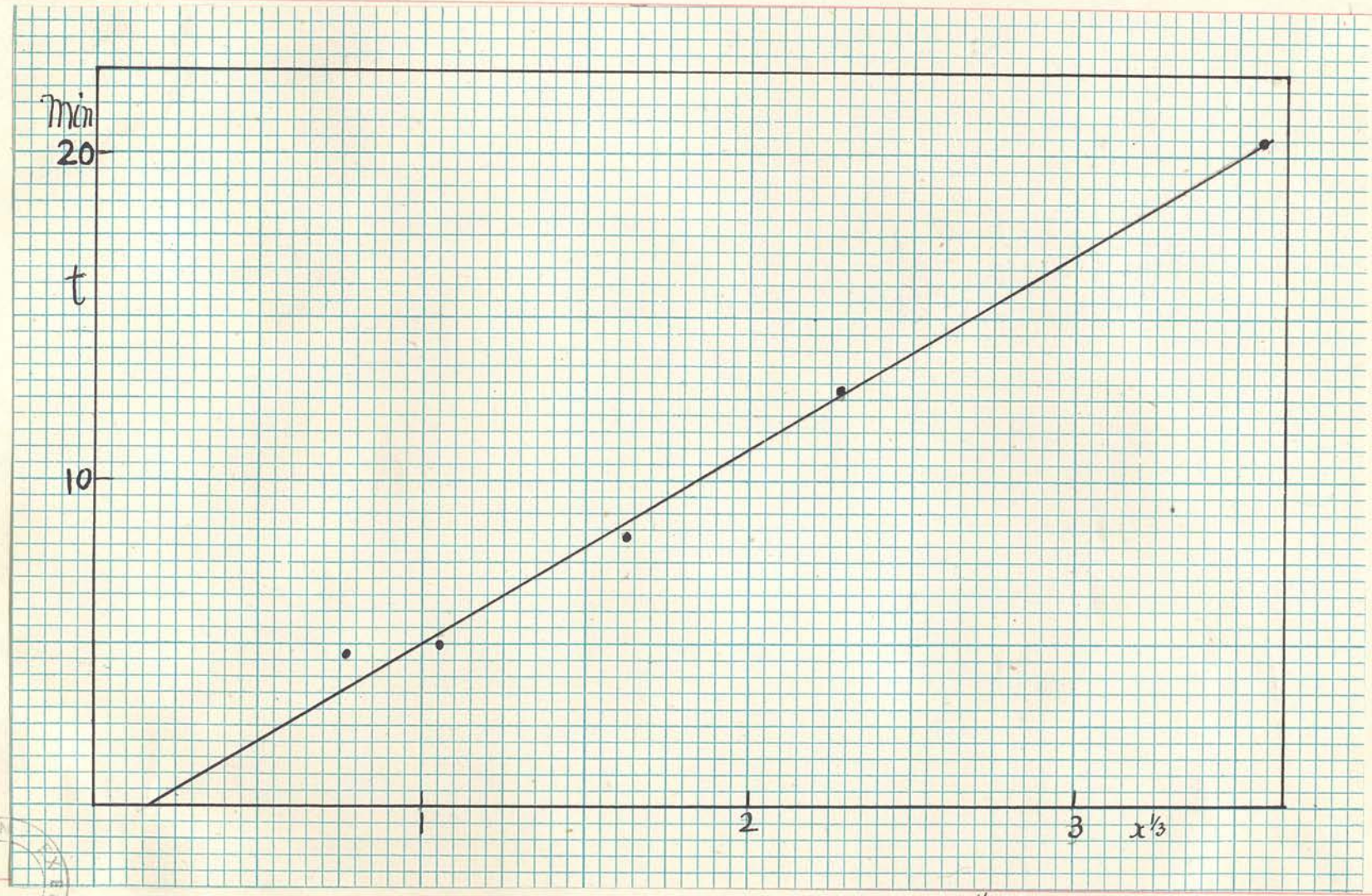


Fig. 11. Relation between adrenaline (dose)$^{1/3}$ µg/kg.(abscissa) and duration of nictitating membrane response in min. (ordinate). Averages of 8 experiments (Table 7)

Fig. 12. Observed and calculated course of response to continuous infusion of adrenaline. Abscissa: time in minutes; ordinates: left, height response n.m. ; right, adrenaline dosage producing corresponding height of response. Continuous line: response produced by 3.4 µg/kg/min. adrenaline for 10 mins.; dotted line: (A) calculated response if no clearance; (B) calculated response according to formula

$$\frac{1}{b - kx^{2/3}}\, dx = dt \quad (k = 0.39)$$

(C) calculated fall according to formula

$$x^{1/3} = x_o^{1/3} - \frac{k}{3}\, t \quad (\frac{k}{3} = 0.13)$$

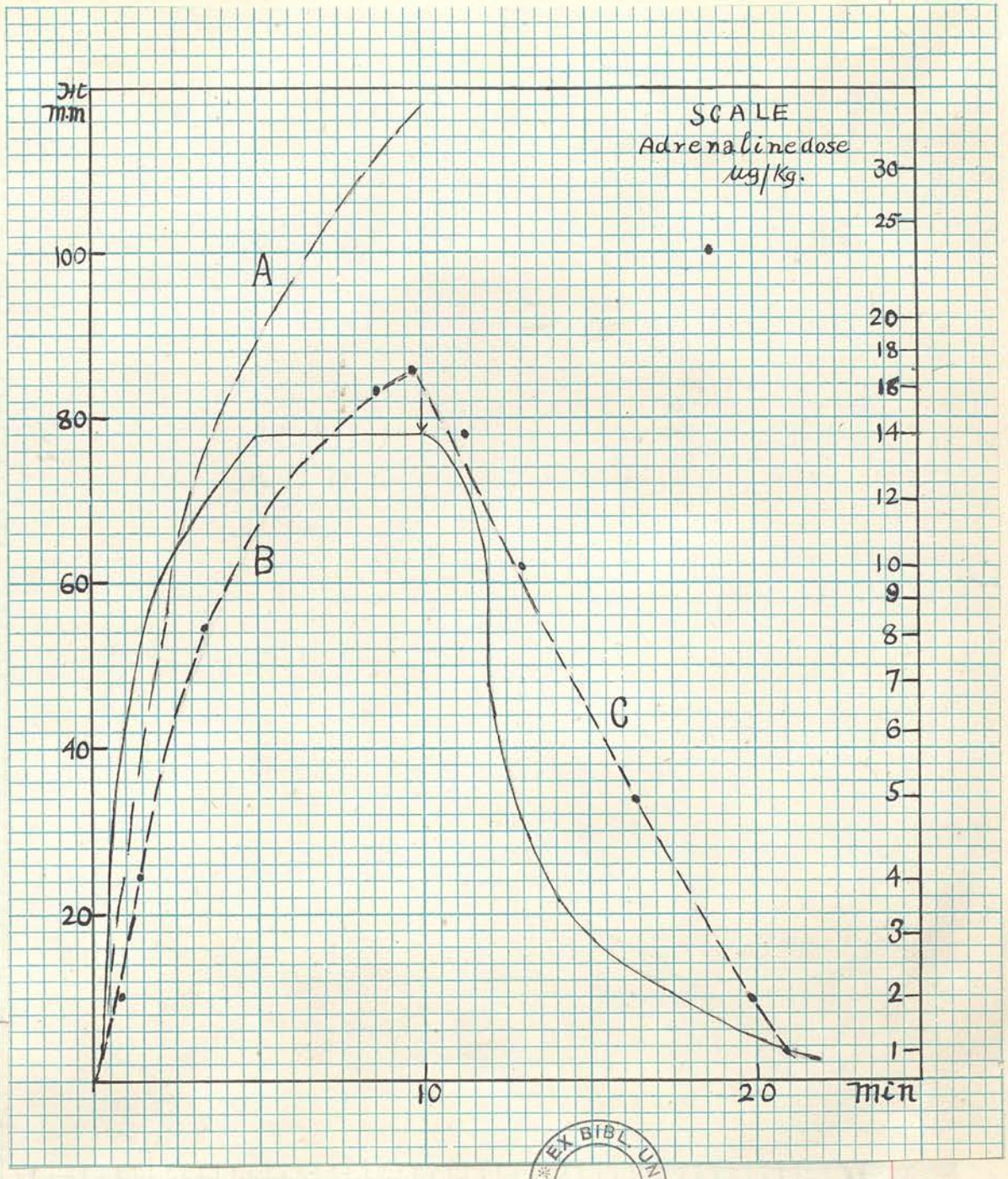


Fig. 12.

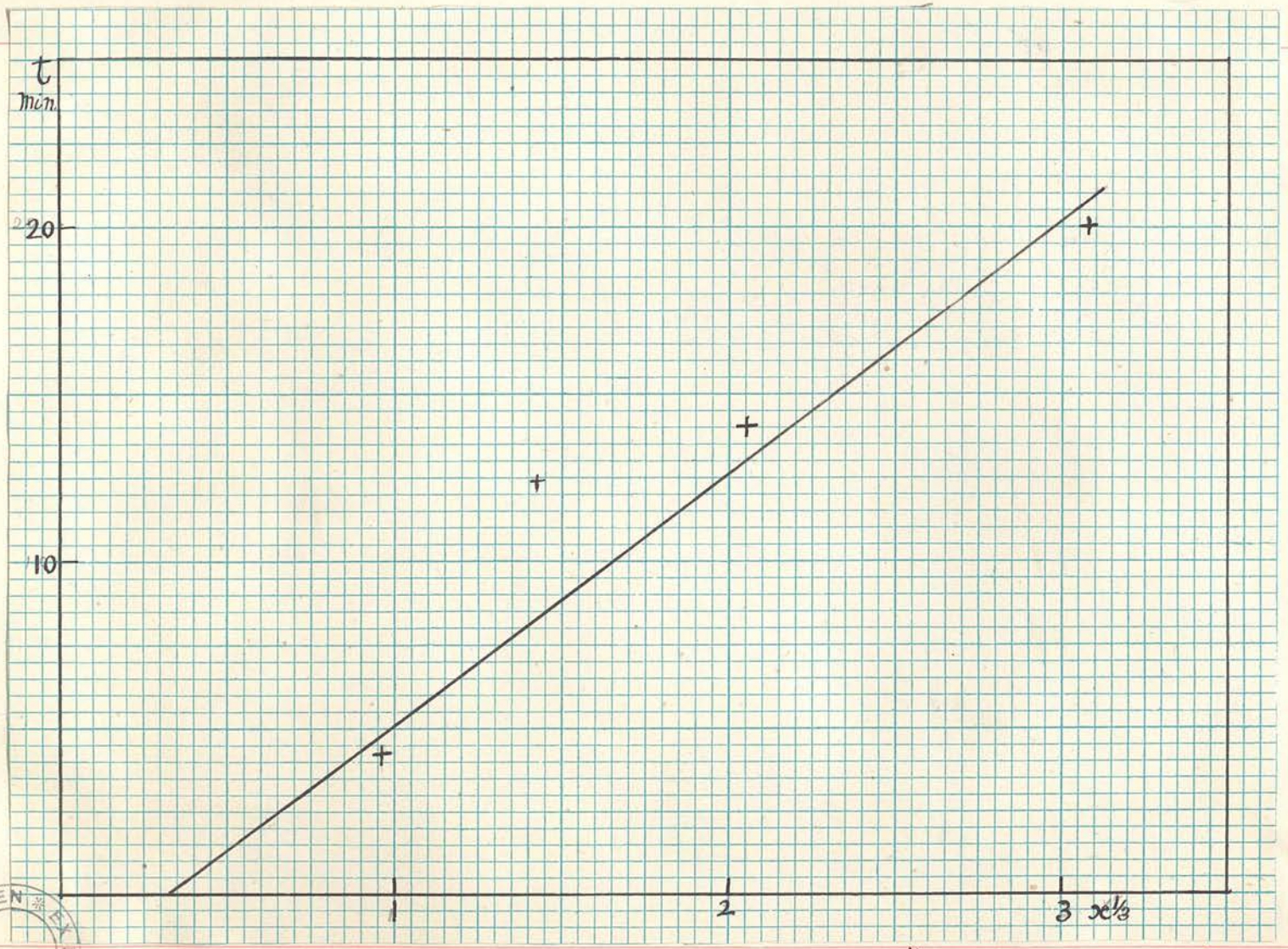


Fig. 13. Relation between adrenaline (dose)$^{1/3}$ µg/kg$^{\frac{1}{3}}$ (abscissa) and duration of n.m. response in min. (ordinate)

Fig. 14. Observed and calculated course of response to a large continuous dose of adrenaline. Abscissa: time in mins. ; Ordinate; left, the height of response n.m.; right, adrenaline dosage producing corresponding height of response. Continuous line: response produced by continuous adrenaline dose of 6.02 µg/kg/min. given for 19 minutes.

Dotted line A: response produced if no clearance; B, calculated response according to formula

$$\frac{1}{b - kx^{2/3}}\, dx = dt$$ where the value of k = 0.51; C, calculated fall according to formula

$$x^{1/3} = x_o^{1/3} - \frac{k}{3} t \quad \left(\frac{k}{3} = 0.17\right)$$

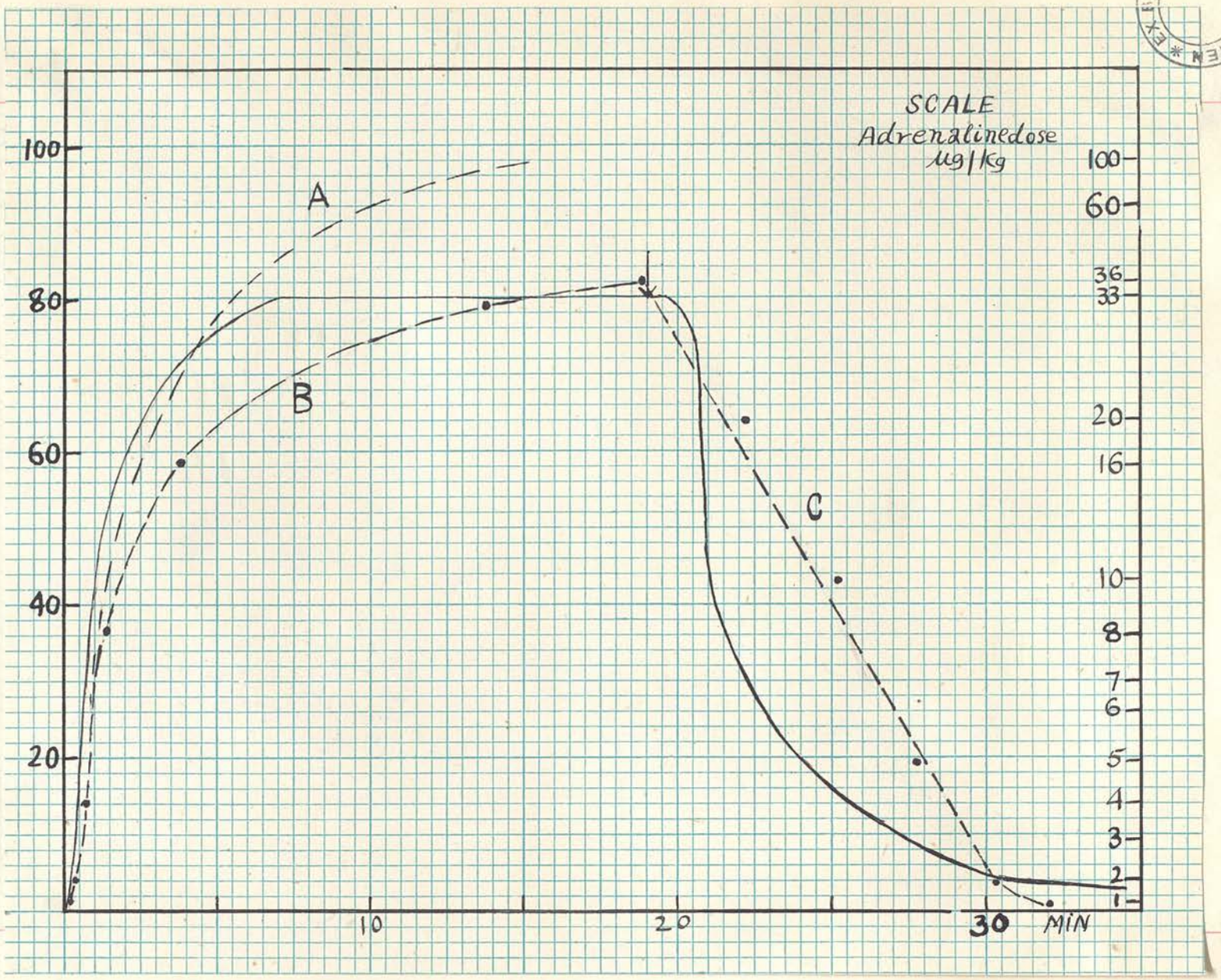


Fig. 14

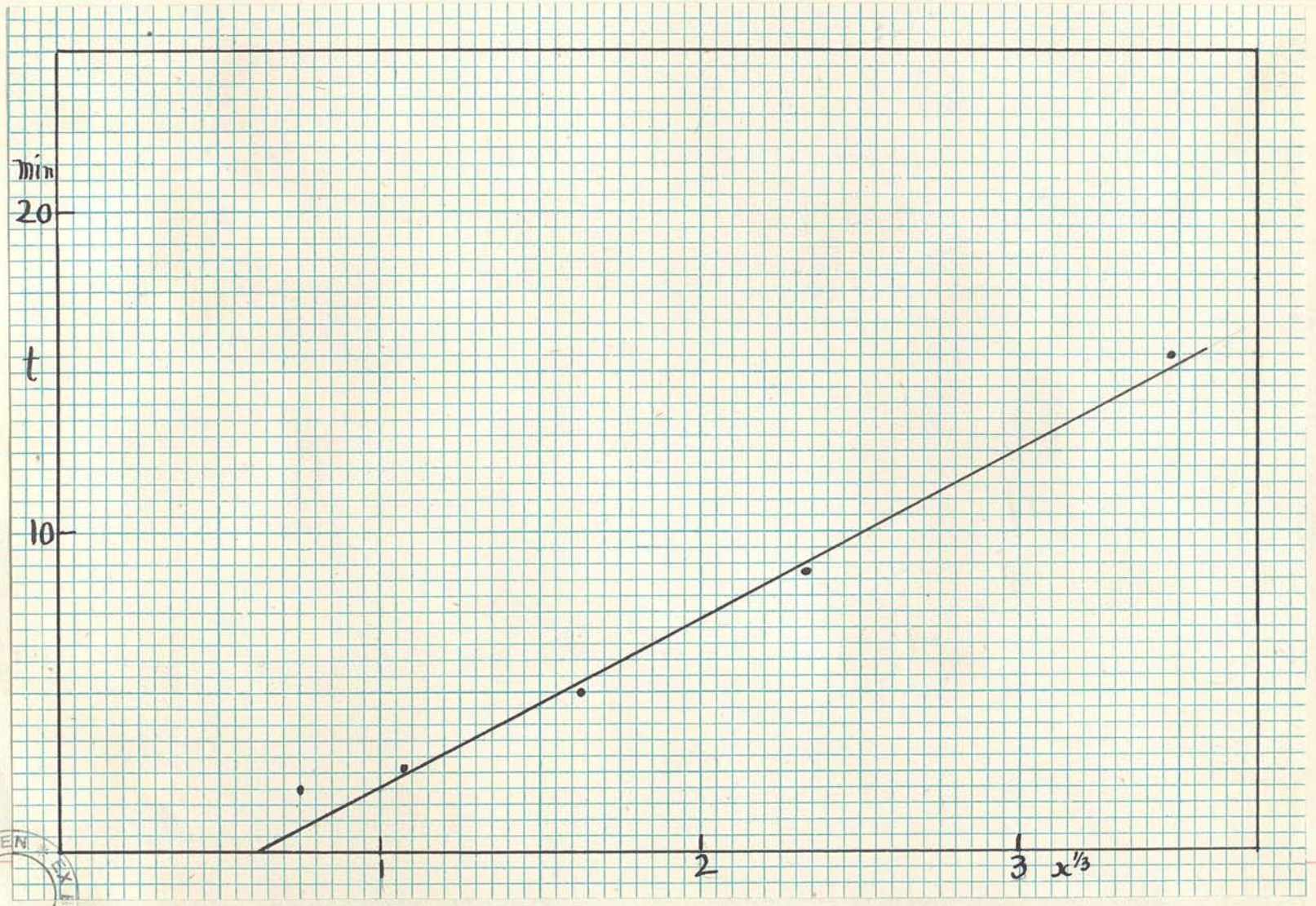


Fig. 15. Relation between adrenaline (dose)$^{1/3}$ µg/kg. (abscissa) and duration of n.m. response in min. (ordinate)

Fig. 16 a. Effect on the n.m. and blood pressure produced by quick adrenaline injection.

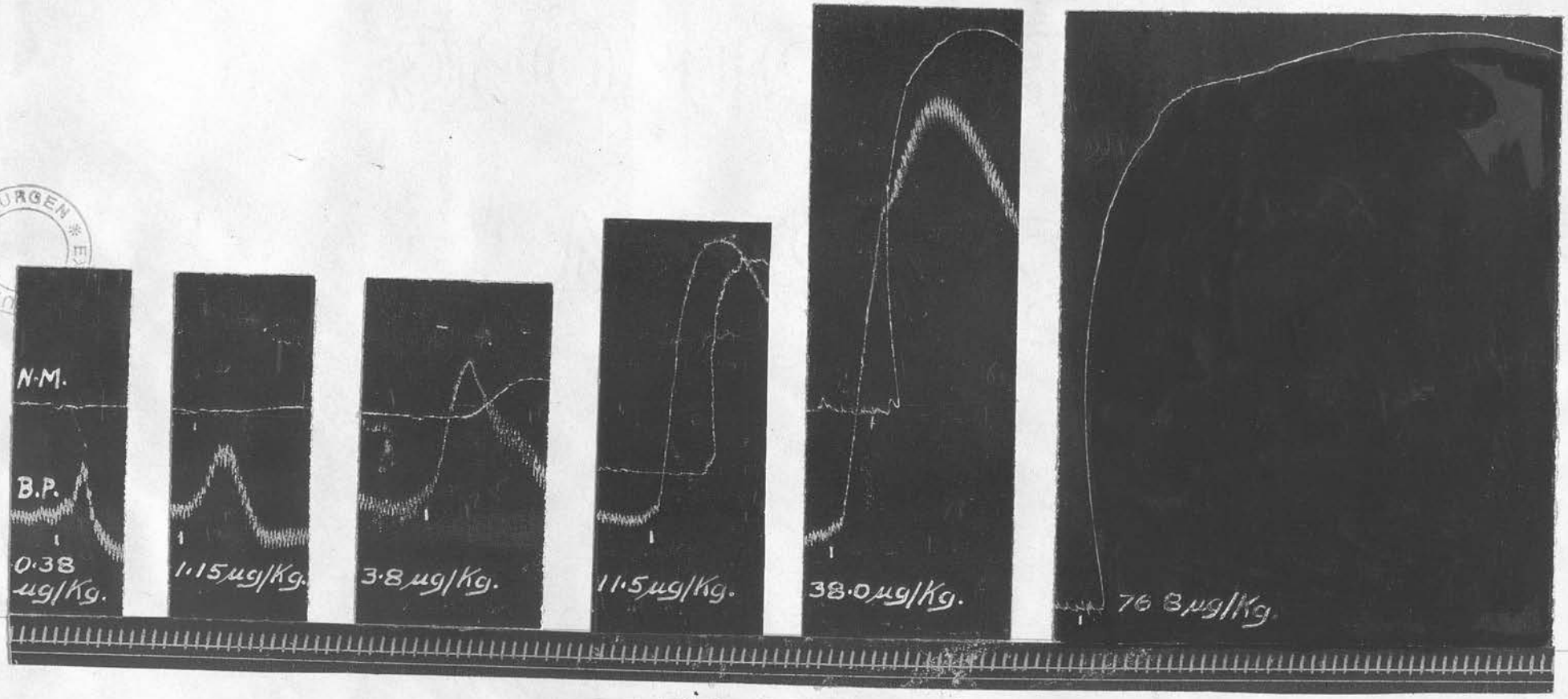


FIG.16a.

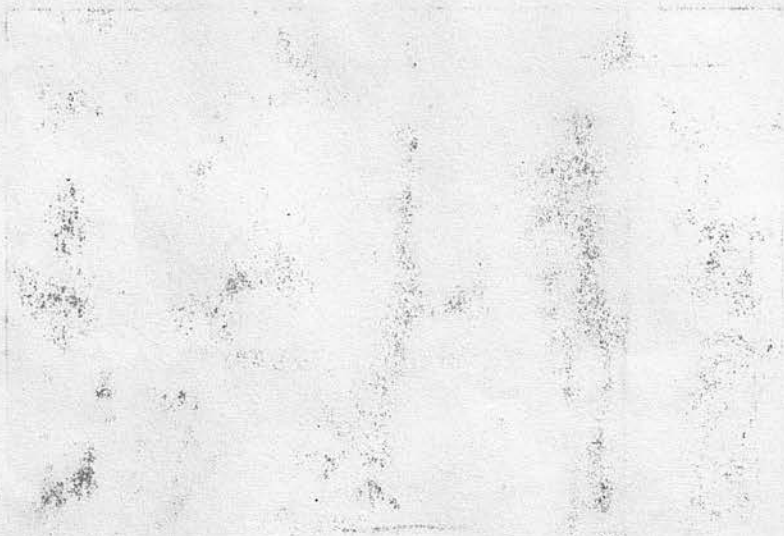
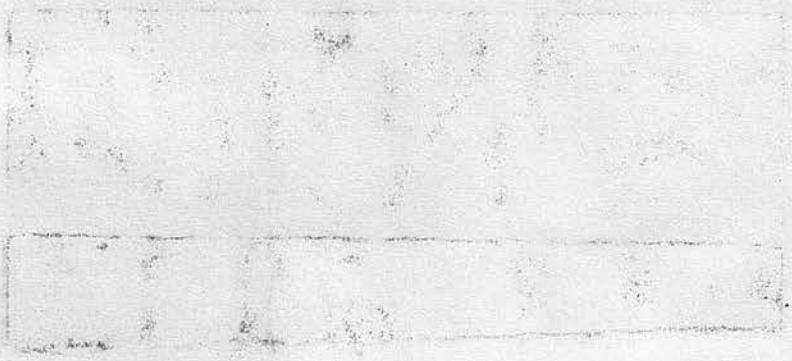


Fig. 16 b_1 . Effects produced by quick adrenaline (11.5 µg/kg.) administered during 0.53 µg/kg/min. continuous infusion of the drug.

Fig. 16 b_2. Effects produced by quick adrenaline (11.5 µg/kg.) administered during 1.79 µg/kg/min. continuous infusion of the drug.



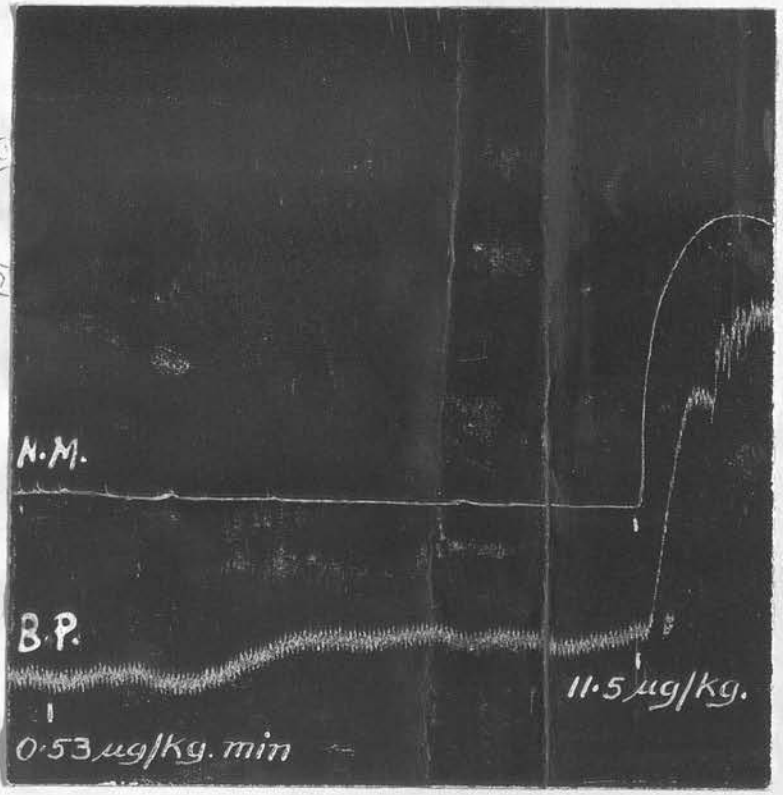


FIG. 16b1.

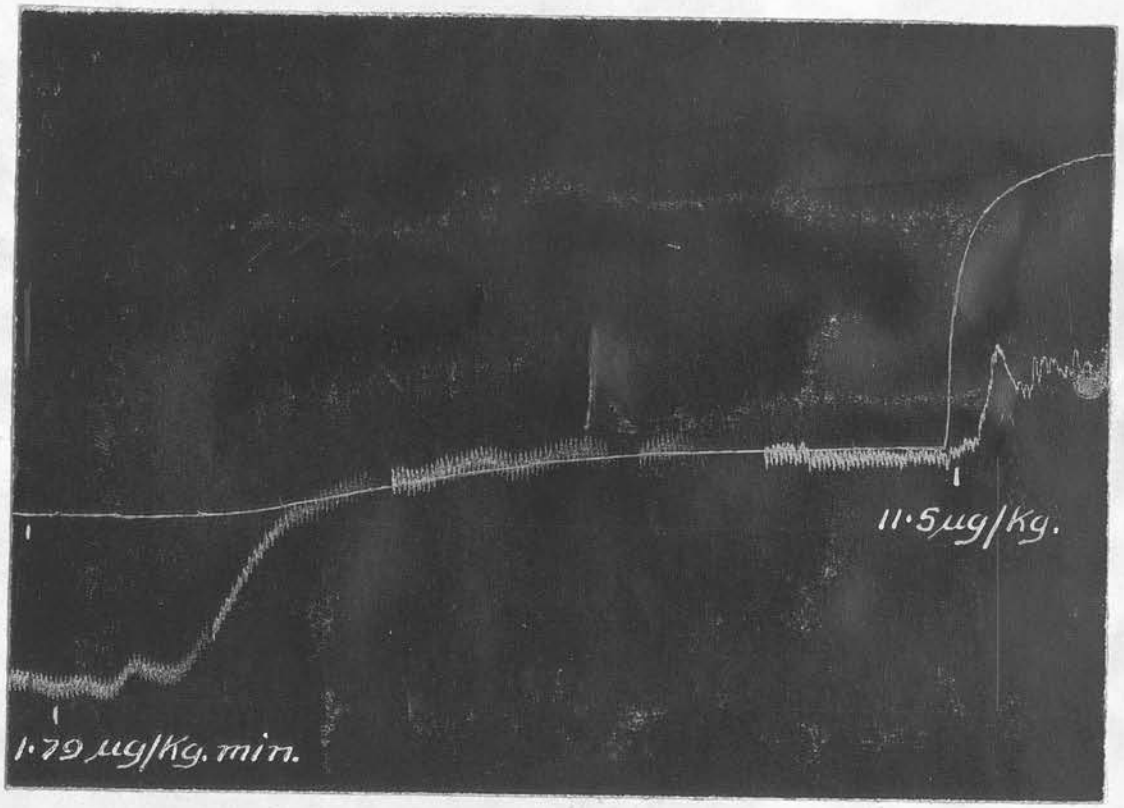


FIG. 16b2.

Fig. 16 c. Effects produced by quick adrenaline (11.5 µg/kg.) administered during 5.3 µg/kg/min. infusion of the drug.

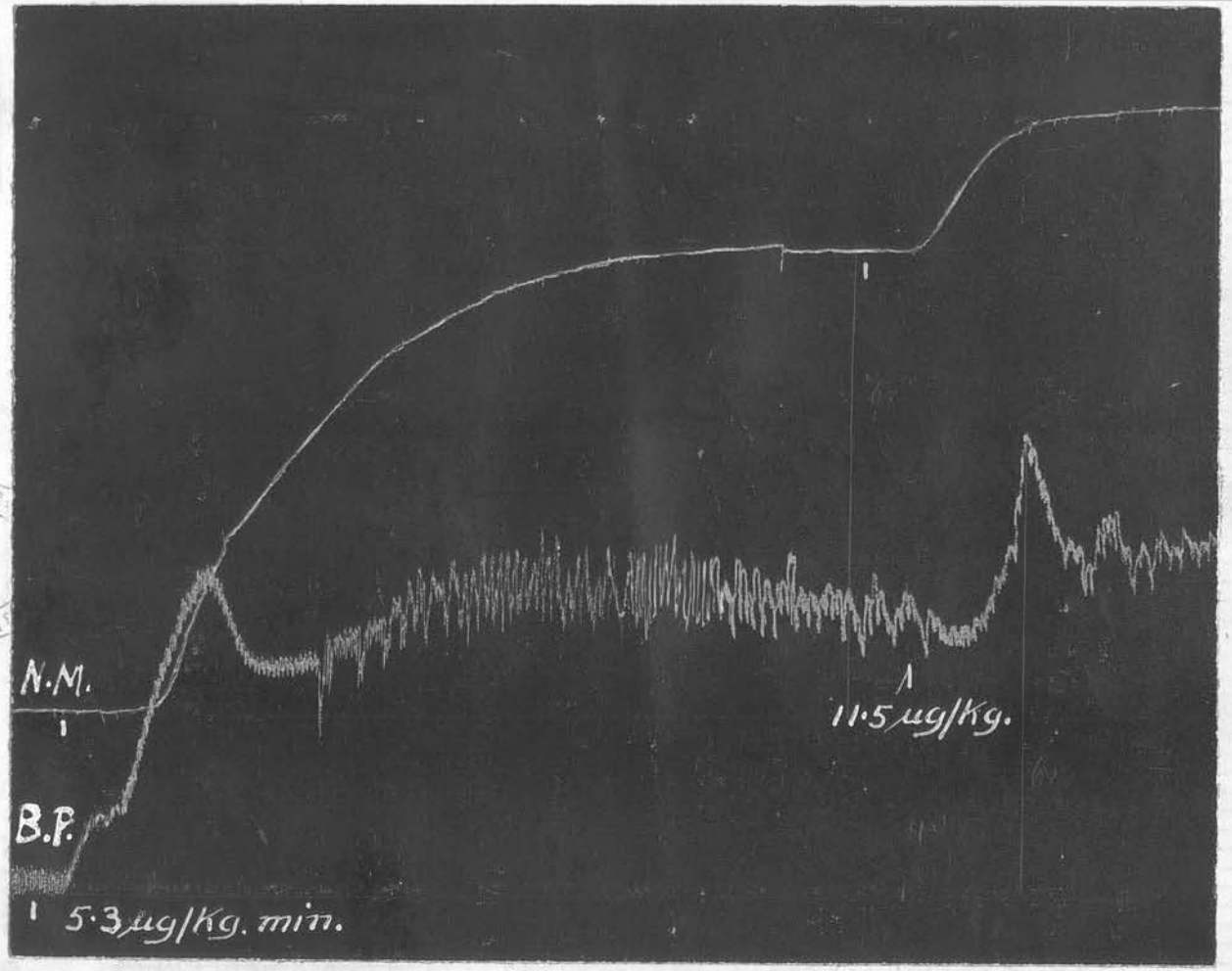


FIG. 16C.

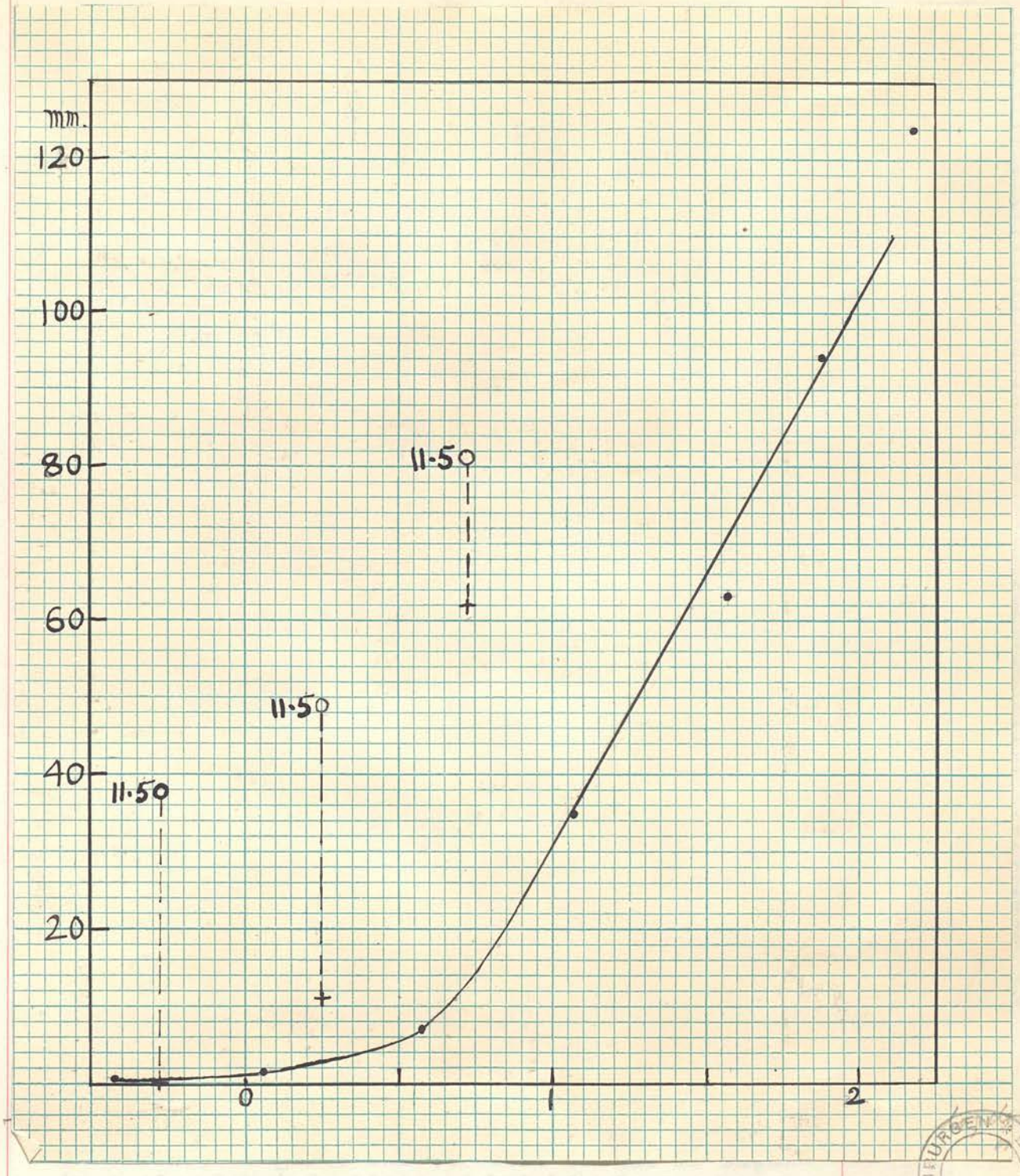


Fig. 17. Effects produced by quick injections of adrenaline superimposed on continuous infusion (from Figs. 16b, b2 and 16c). Abscissa: log. dose µg/kg. or µg/kg./min. Ordinate: height of response of n.m. Continuous line and dots: dosage response curve to quick injections; crosses: response to continuous injection; figures and circles: amount of superimposed quick injection and height of response.

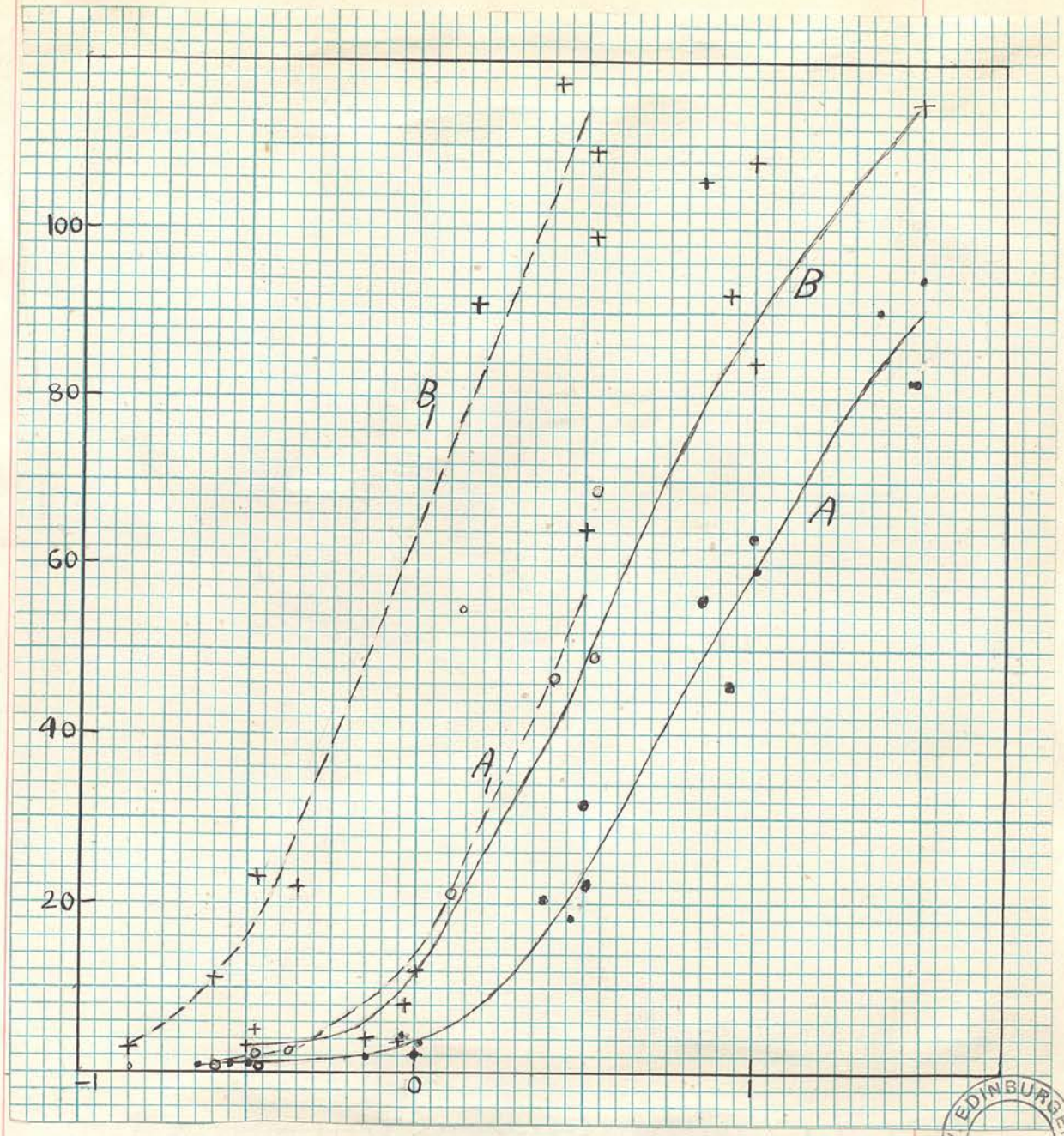


Fig. 19. Effects produced by quick doses of adrenaline. A, before pyrogallol; B, after pyrogallol, and continuous adrenaline before pyrogallol A_1, and after pyrogallol B_1, from 4 experiments.
Abscissa: log. dose µg/kg. or µg/kg/min.
Ordinate: height of response of n.m.

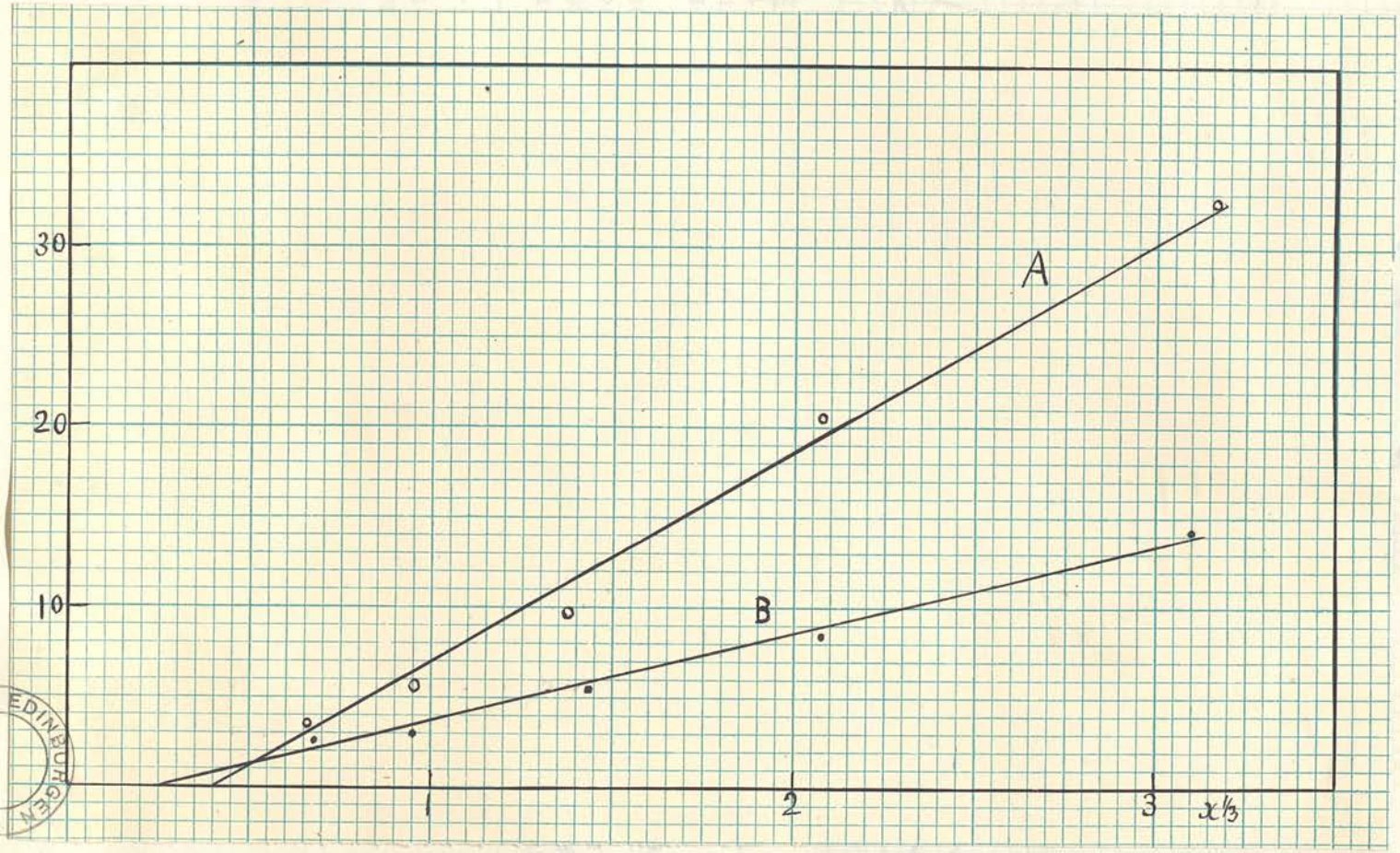


Fig. 21. Relation between (adrenaline dose) µg/kg.(abscissa) and duration of n.m. response in min.(ordinate). B: before pyrogallol; A: after pyrogallol (From the averages of 7 experiments, Table 12)

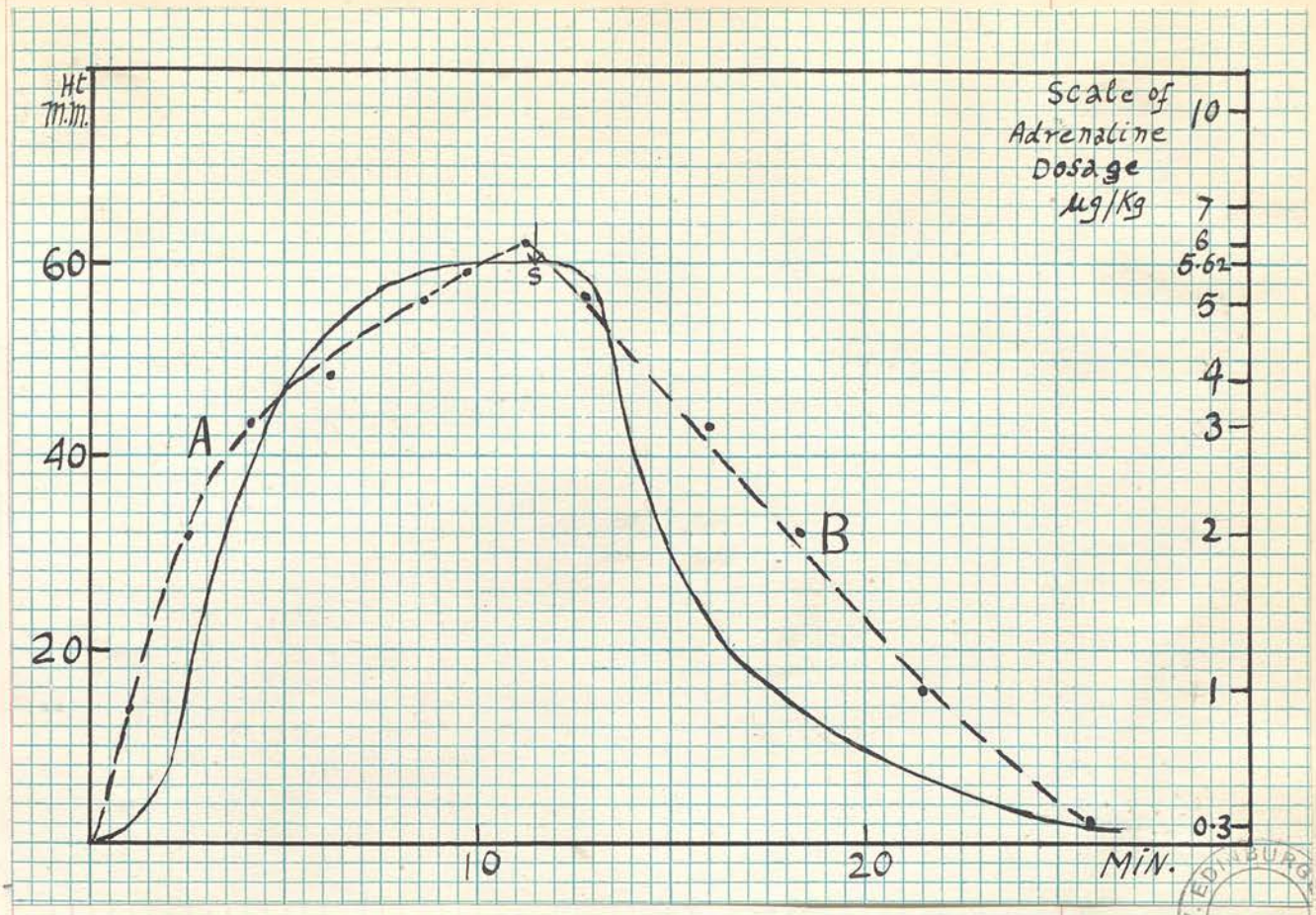


Fig. 22. Observed course of n.m. response and calculated cumulation and clearance after pyrogallol administration.
Abscissa: time in min.; ordinates: left, height of response of n.m. in mm., right, adrenaline dosage producing corresponding heights of response after pyrogallol; continuous line, response produced by 1.09 µg/kg/min. adrenaline after pyrogallol; dotted line (A), calculated cumulation by means of formula

$$\frac{1}{b - kx^{2/3}}\,dx = dt$$ where value of k = 0.24.

Dotted line B_1, calculated clearance by means of formula:

$$x^{1/3} = x_0^{1/3} - \frac{k}{3}\,t$$

where $\frac{k}{3} = 0.08$.

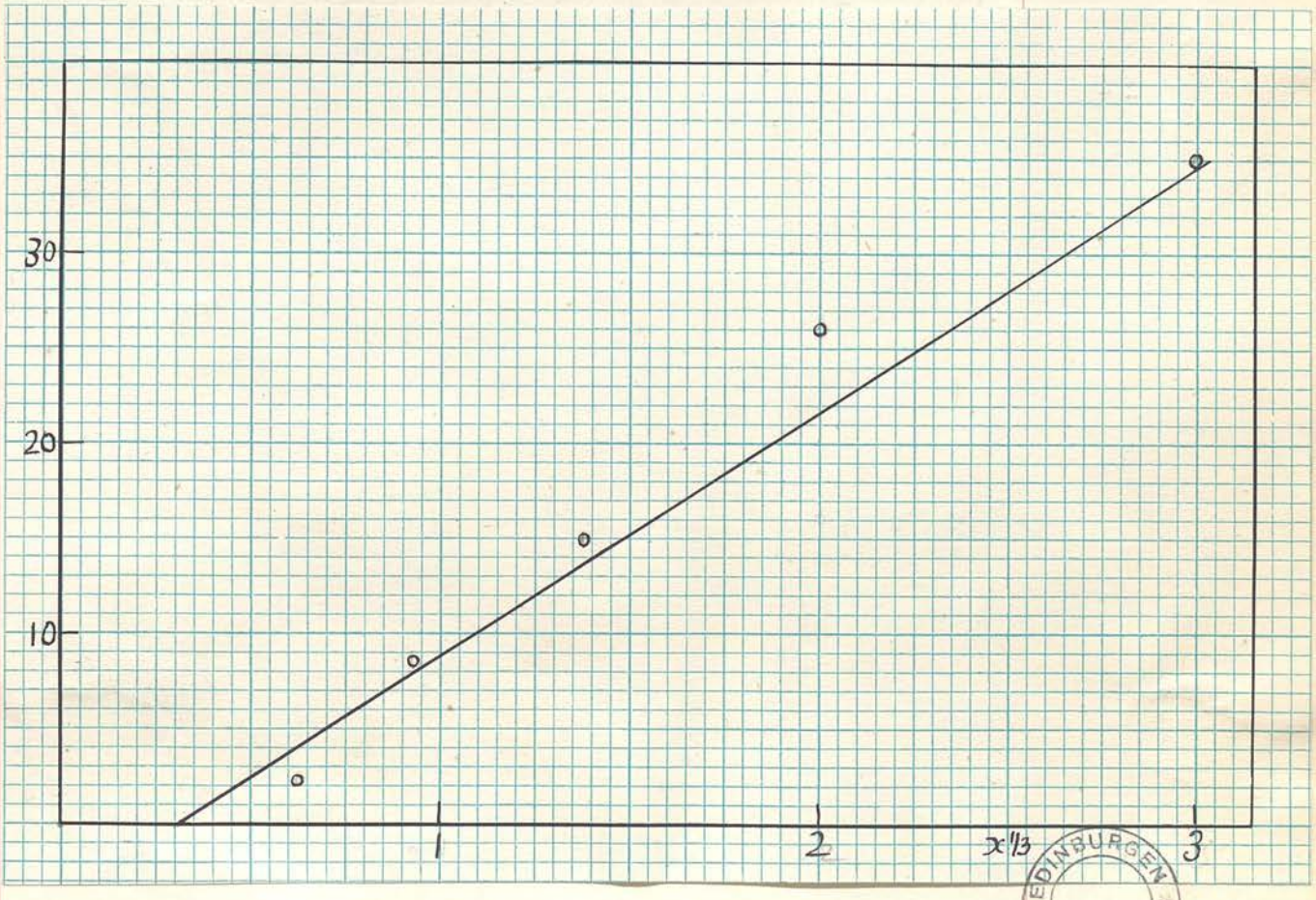


Fig. 23. Relation between (adrenaline dose)$^{1/3}$ µg/kg. (abscissa) and duration of n.m. response in min. (ordinate)

Fig. 24. Observed and calculated course of response to continuous adrenaline injection before and after pyrogallol.

Continuous line (A): response n.m. to continuous 1.48 µg/kg/min. adrenaline before pyrogallol; continuous line A_1 response to the same dose after pyrogallol.

Dotted line B and B_1 calculated cumulation before and after pyrogallol; C and C_1 calculated fall before and after pyrogallol. Value of $\frac{k}{3}$ = 0.09 (after pyrogallol and 0.18 before pyrogallol).

Abscissa: time in minutes; ordinates: left, height of n.m. response in mm; right I and II, adrenaline dosages (µg/kg) before and after pyrogallol respectively producing corresponding height of response.

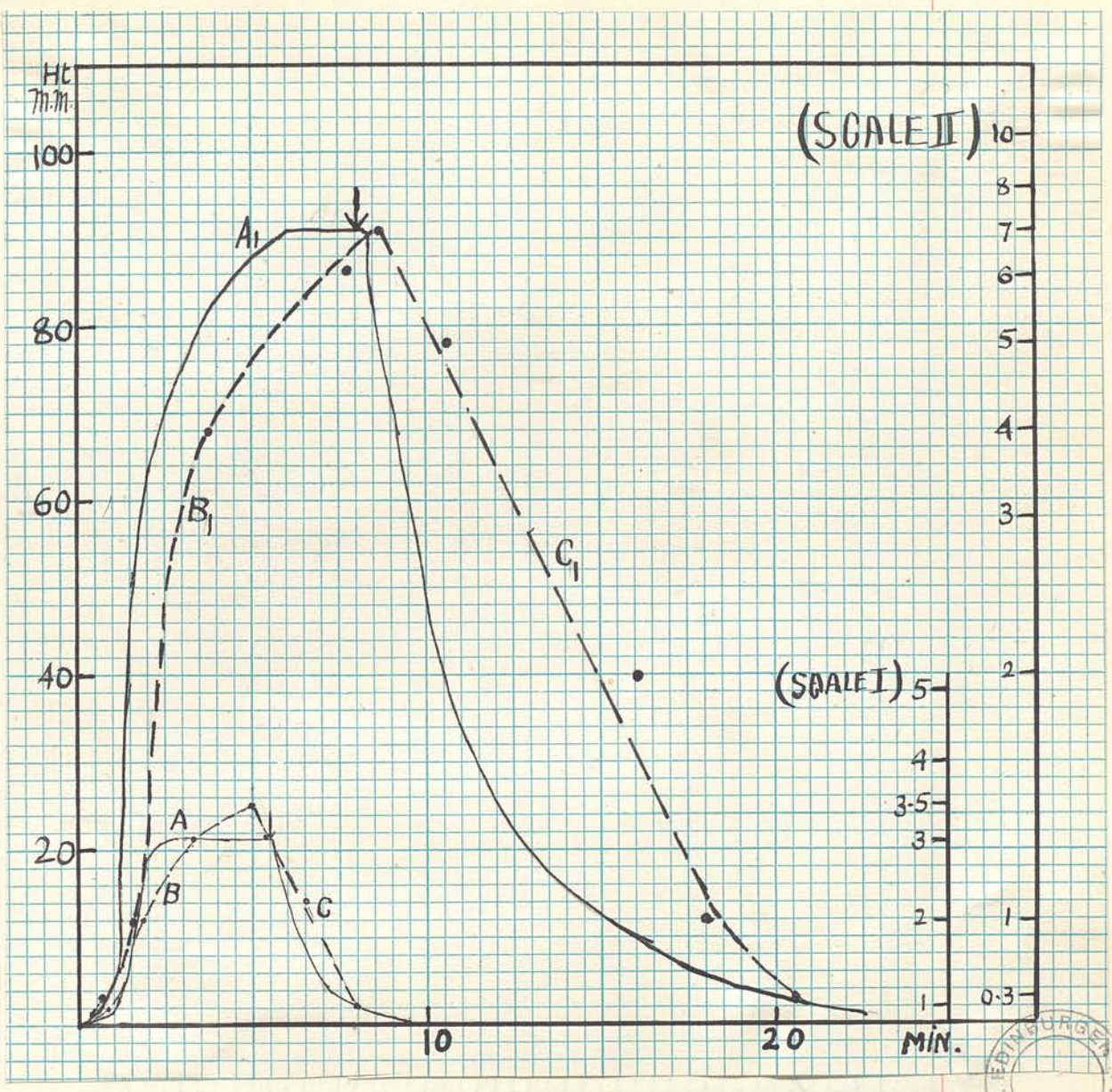

Ht
m.m.
100
80
60
40
20
A1
B1
C1
A
B
C
(SCALE II)
10
8
7
6
5
4
3
2
1
0·3
(SCALE I)
5
4
3·5
3
2
1
10
20
MIN.

Fig. 24.

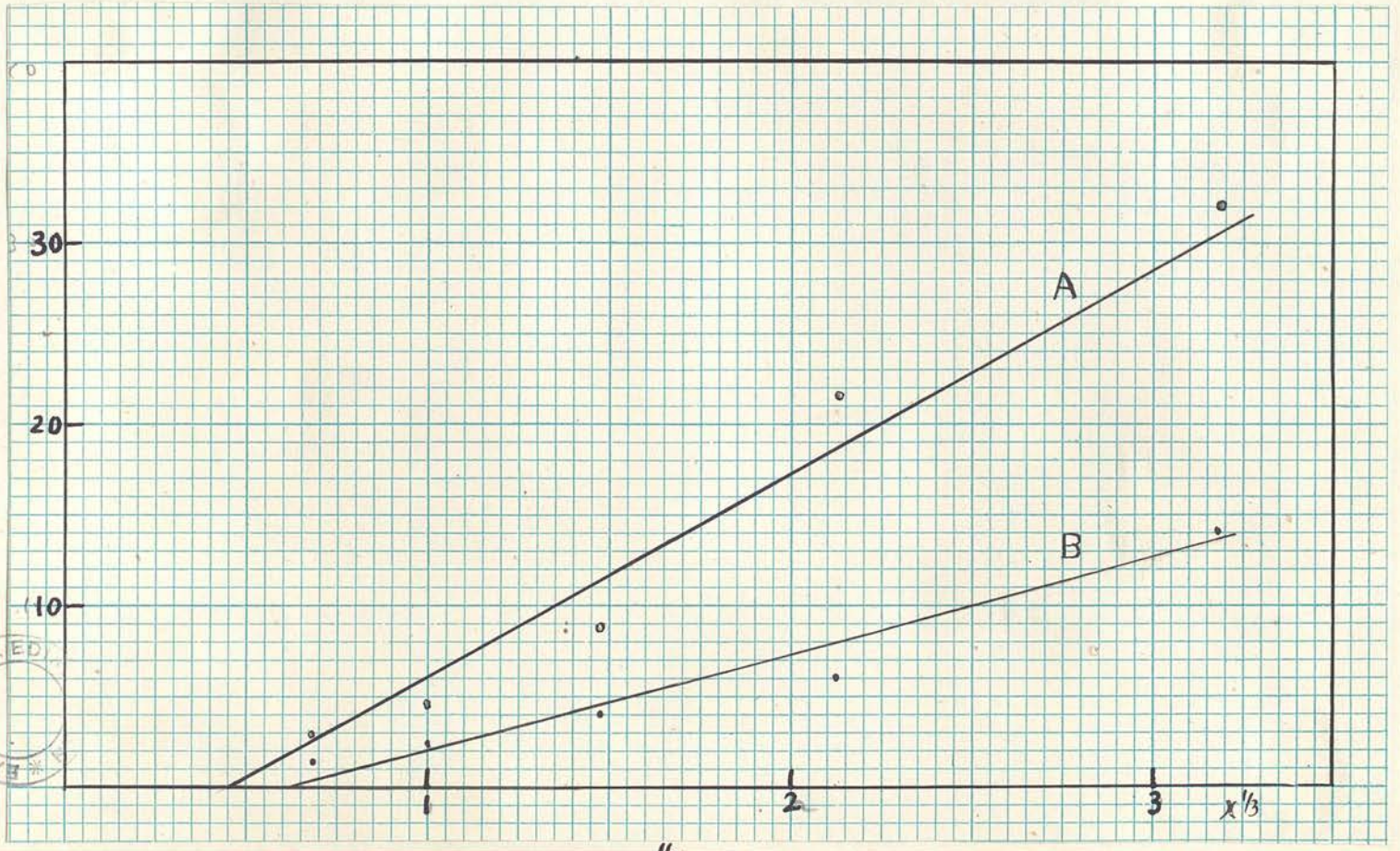


Fig. 25. Ratio between (adrenaline dose)$^{1/3}$ µg/kg.(abscissa) and duration of n.m. response in min. (ordinate)

Fig. 26 The response of the n.m. and blood pressure to an intravenous cocaine dose of 2.5 mg/kg.

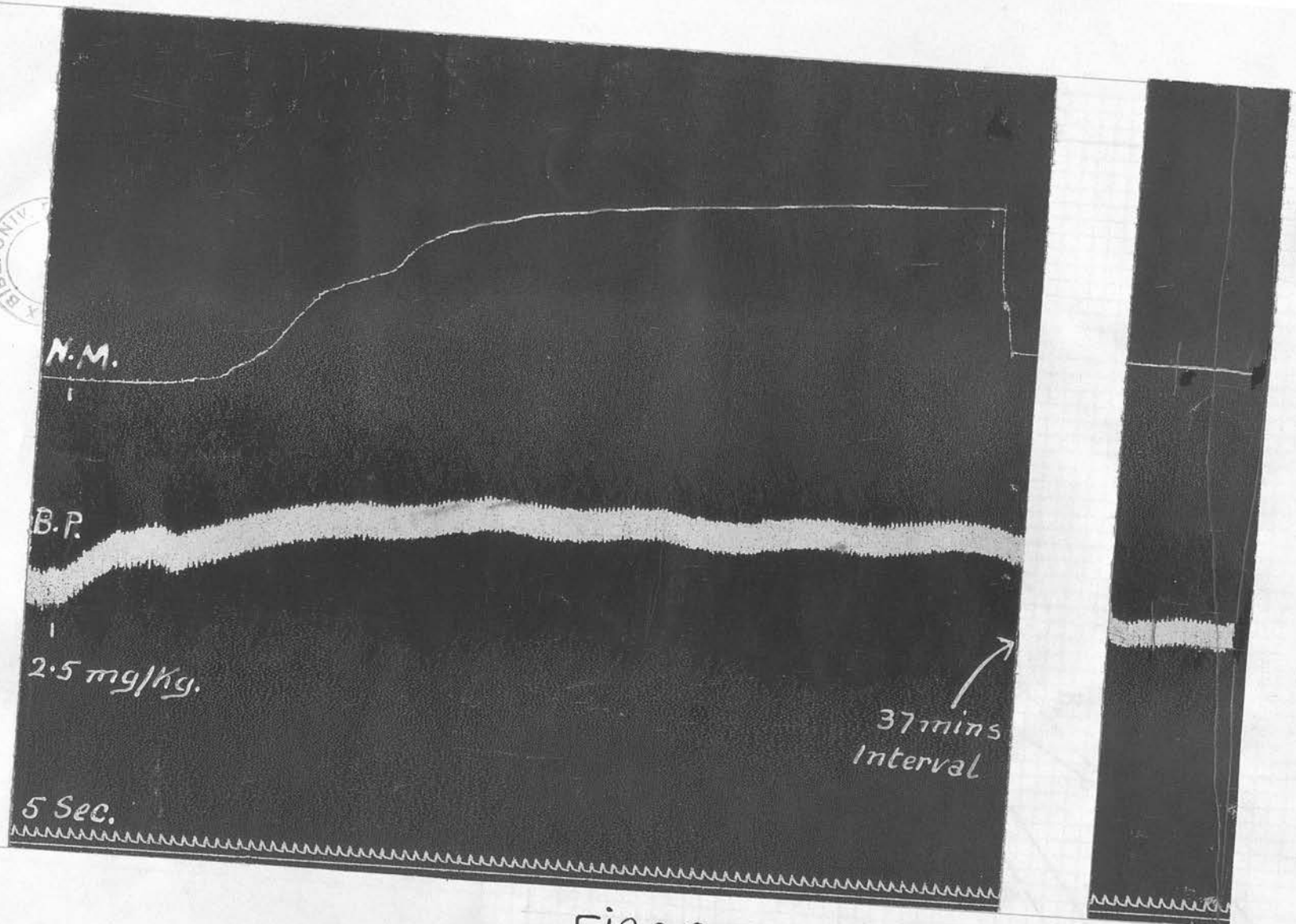


FIG.26.

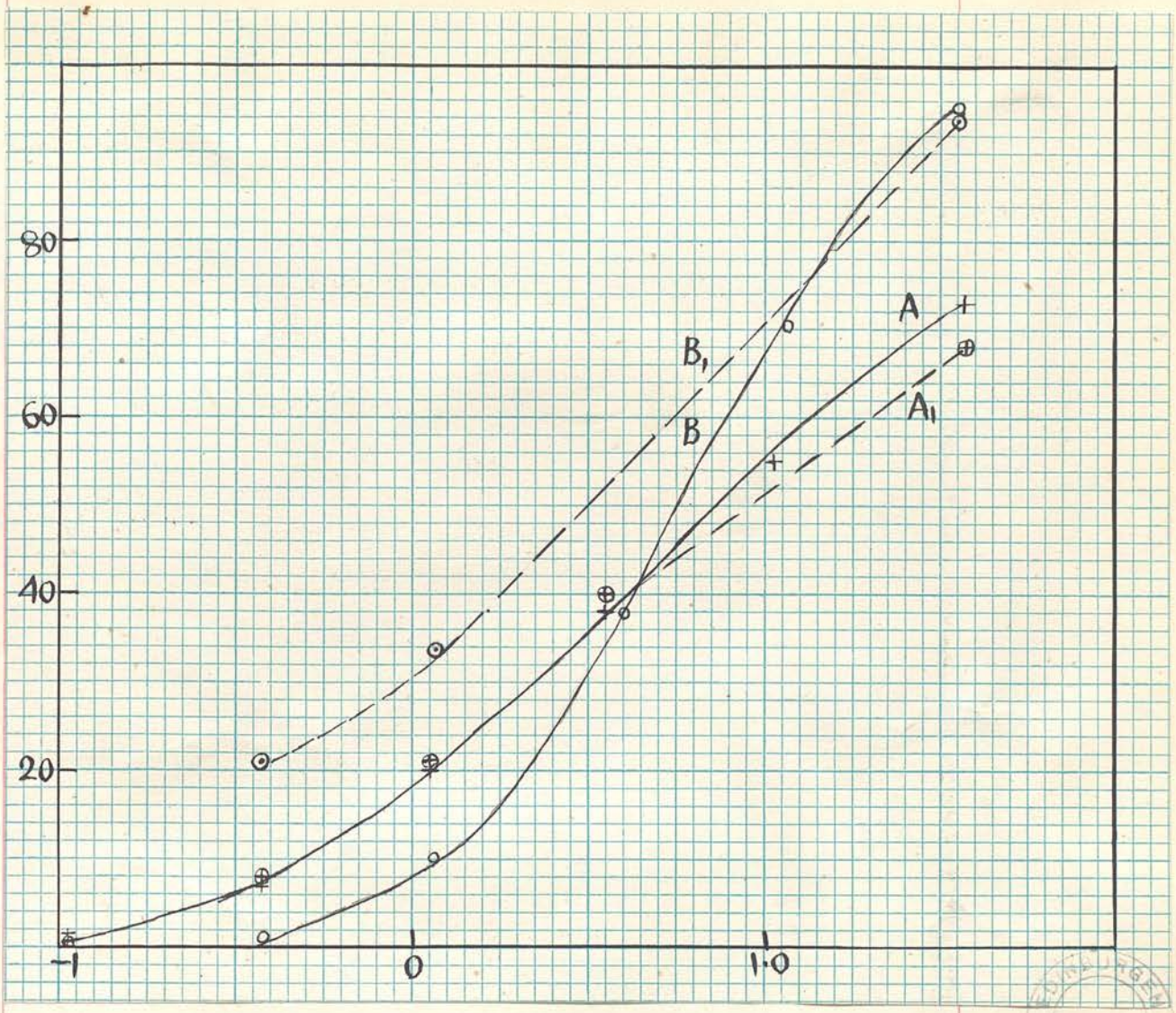


Fig. 27. Effect of large doses of cocaine on the sensitivity of the n.m. response to adrenaline.

Abscissa: log. dose adrenaline µg/kg.
Ordinate: height of n.m. response in mm.
Curves B and B_1, responses of the n.m. before and after 3.9 mg/kg. intravenous cocaine administration.
A and A_1, responses of the n.m. before and after 5.2 mg/kg. cocaine.

Fig. 28. Relation between log. dosage of quick and continuous adrenaline and n.m. response before and after cocaine (from 3 experiments).

Abscissa: log. dose µg/kg. or µg/kg/min.; ordinate, height of n.m. response.

A and A_1, single and continuous adrenaline before cocaine.

B and B_1, do. do. after cocaine.

(Dose of cocaine ranged from 1.5 to 3 mg/kg.

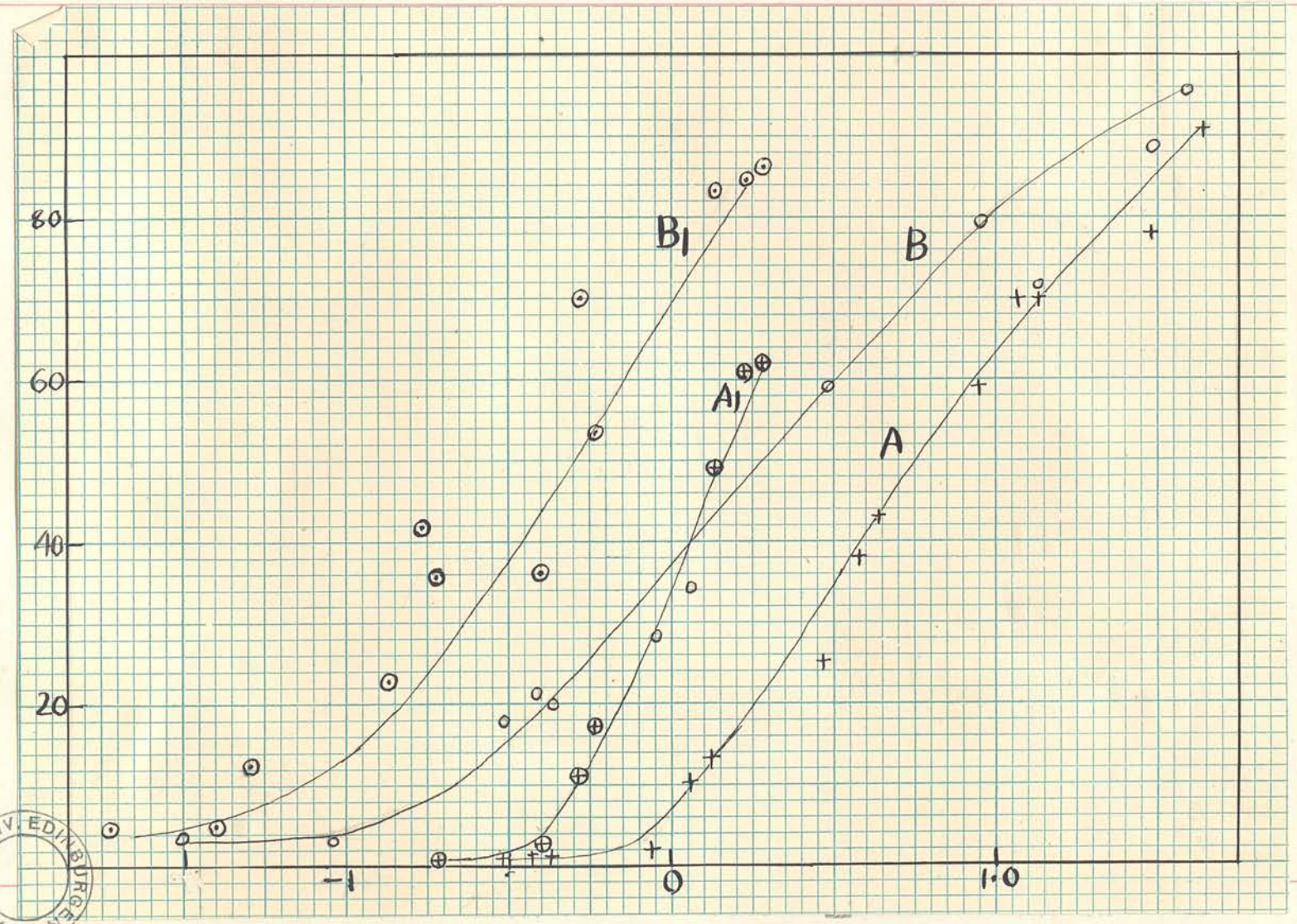


Fig. 28.

Fig. 29. Relation between log. dosage of quick and continuous adrenaline and blood pressure response before and after cocaine (from 7 experiments).

Abscissa: log. dose µg/kg. or µg/kg/min.; ordinate: height of blood pressure response in mm.

A and A_1, single and continuous adrenaline before cocaine.

B and B_1, single and continuous adrenaline after cocaine (dose of cocaine ranged from 1.5 to 3 mg/kg.)

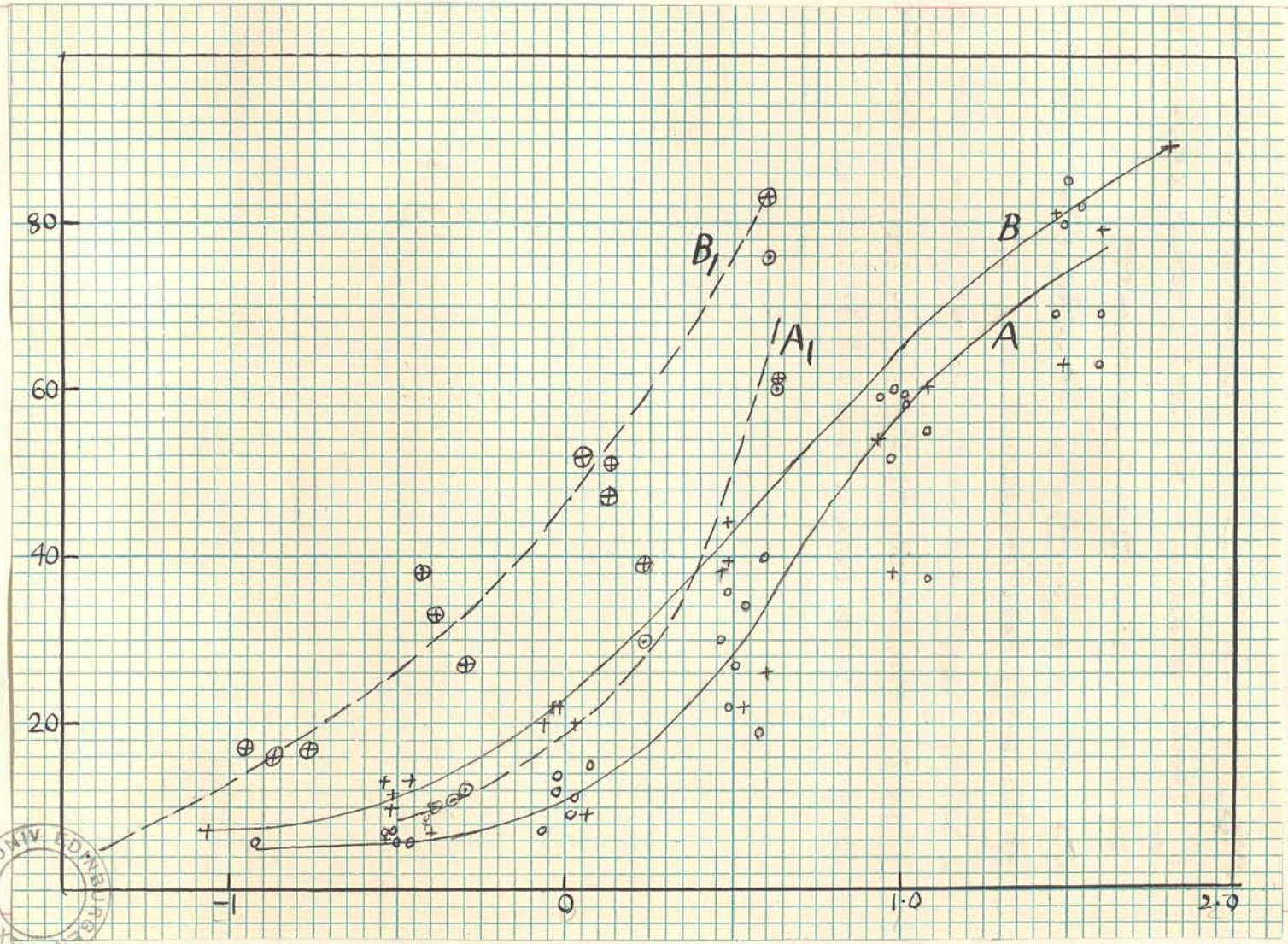


Fig. 29.

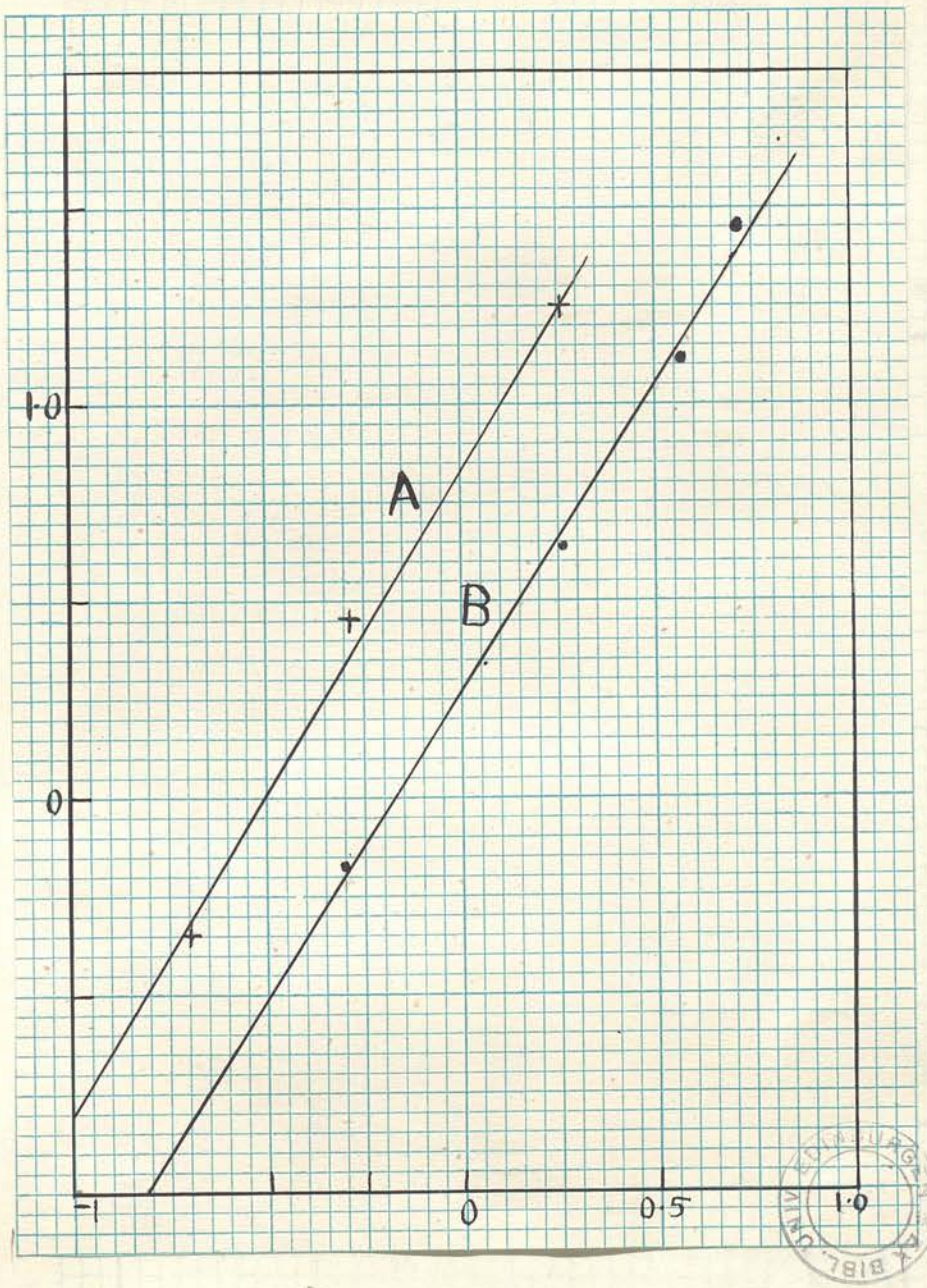


Fig. 30. Relation between continuous dose µg/kg/min. (abscissa) and quick dose µg/kg. (ordinate) before cocaine (B) and after cocaine (A). (from averages of 5 experiments with n.m.)

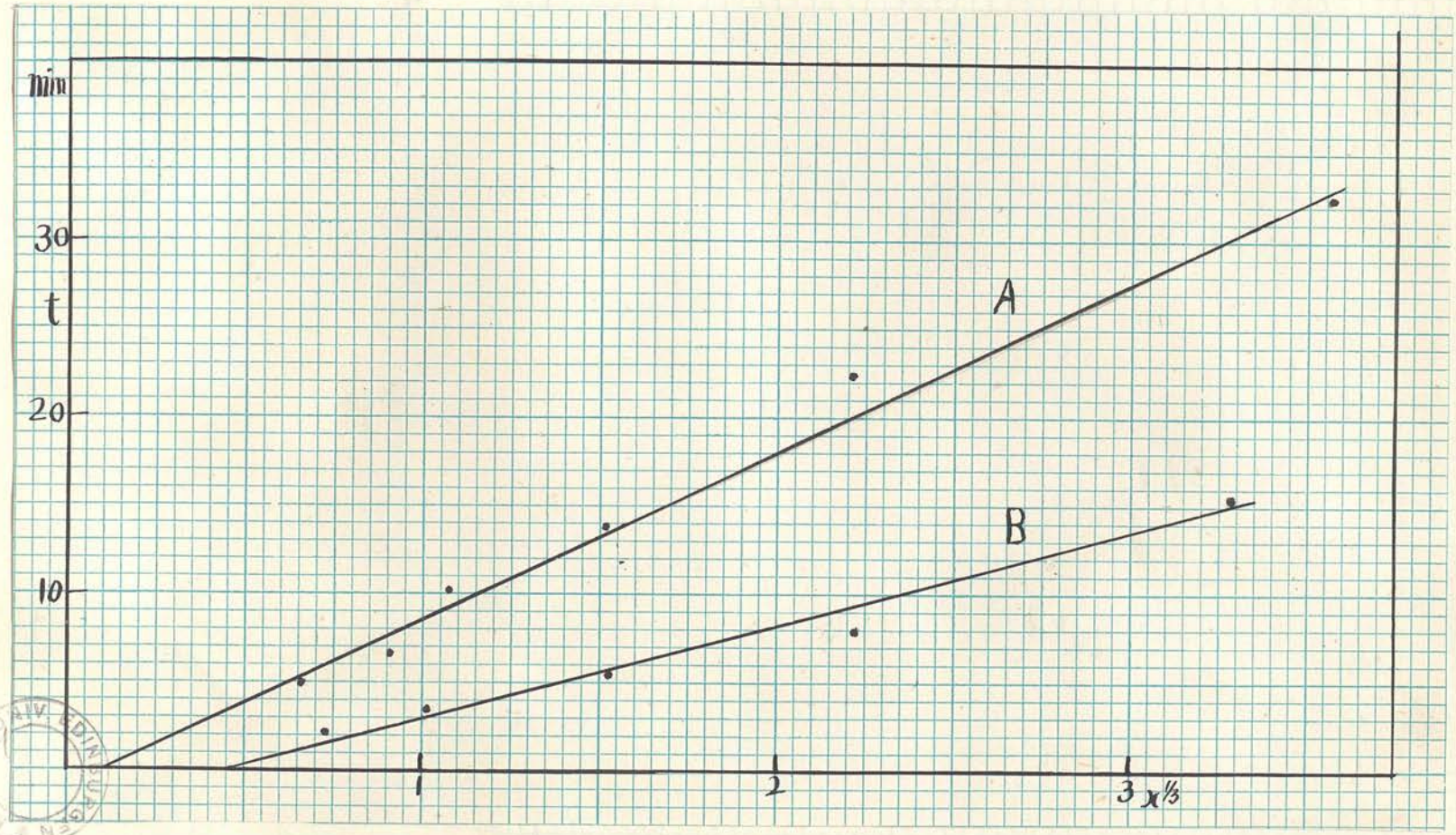


Fig. 31. Relation between (dosage adrenaline)$^{1/3}$ µg/kg.(abscissa) and duration of n.m. response in mins.(rodinate). B, before cocaine and A, after cocaine (from averages of 8 experiments)

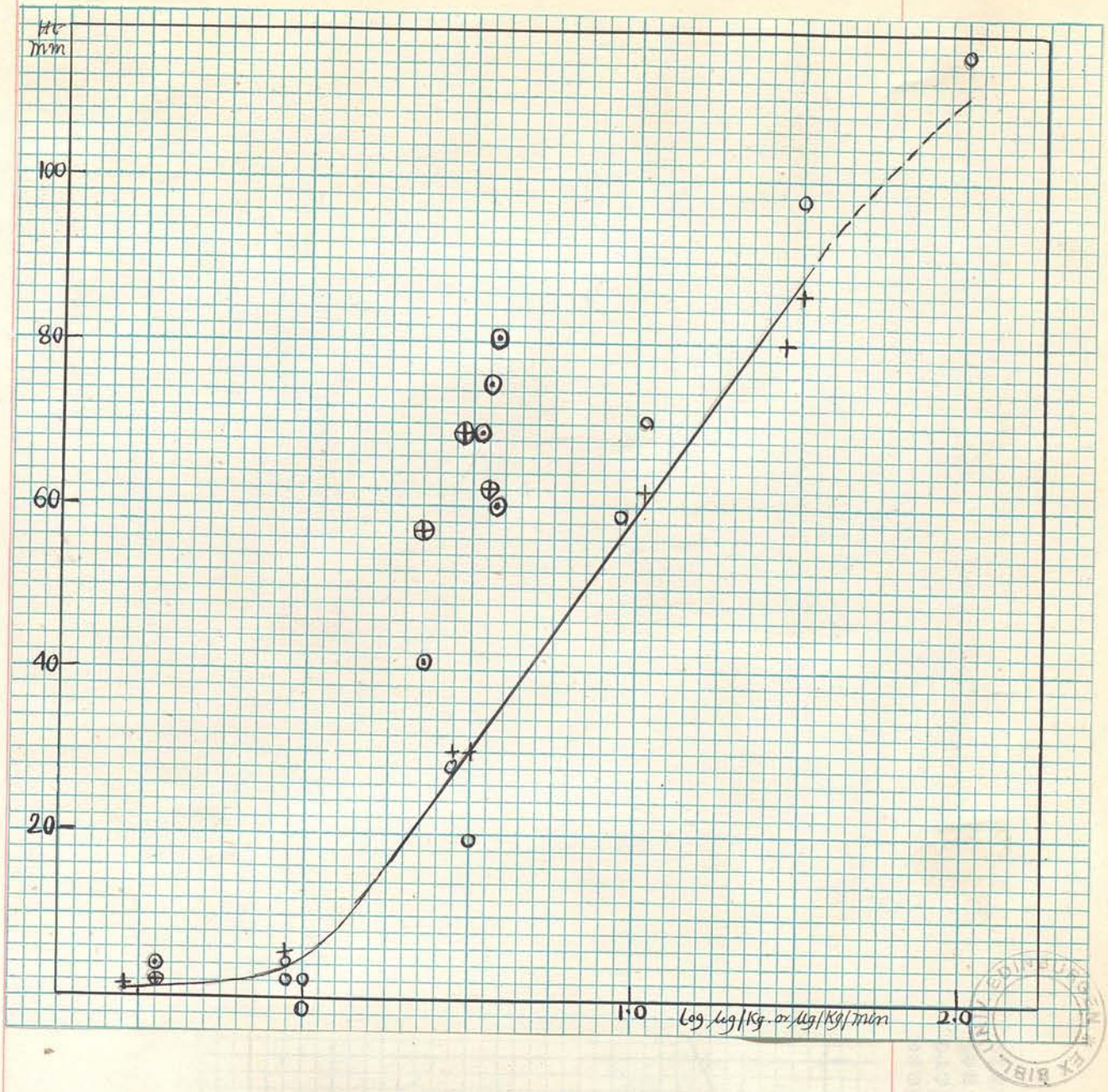


Fig. 32. Relation between log. dosage of quick and continuous adrenaline and n.m. response before and after ascorbic acid administration.

+ = quick adrenaline before ascorbic acid.
O = do. after do.
⊕ = continuous adrenaline before ascorbic acid.
⊙ = do. after do.

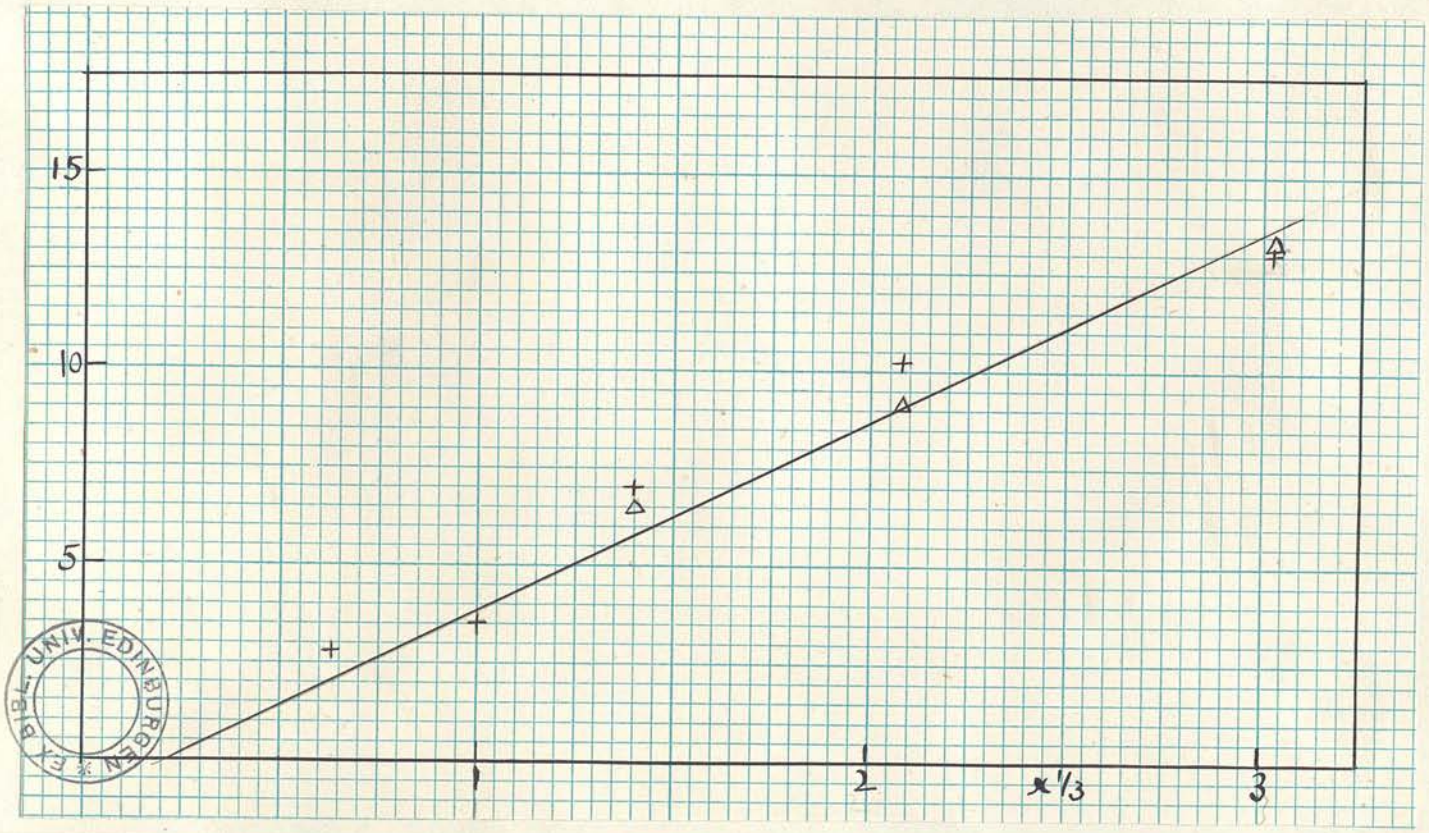


Fig. 33. Relation between (dosage) adrenaline µg/kg. (abscissa) and duration of n.m. response in minutes (ordinate).

+ Before ascorbic acid,

Δ After ascorbic acid.

Fig. 34a. Responses of the n.m. and blood pressure of cat to continuous intravenous infusions of 5.53 µg/kg/min. adrenaline before ascorbic acid administration.

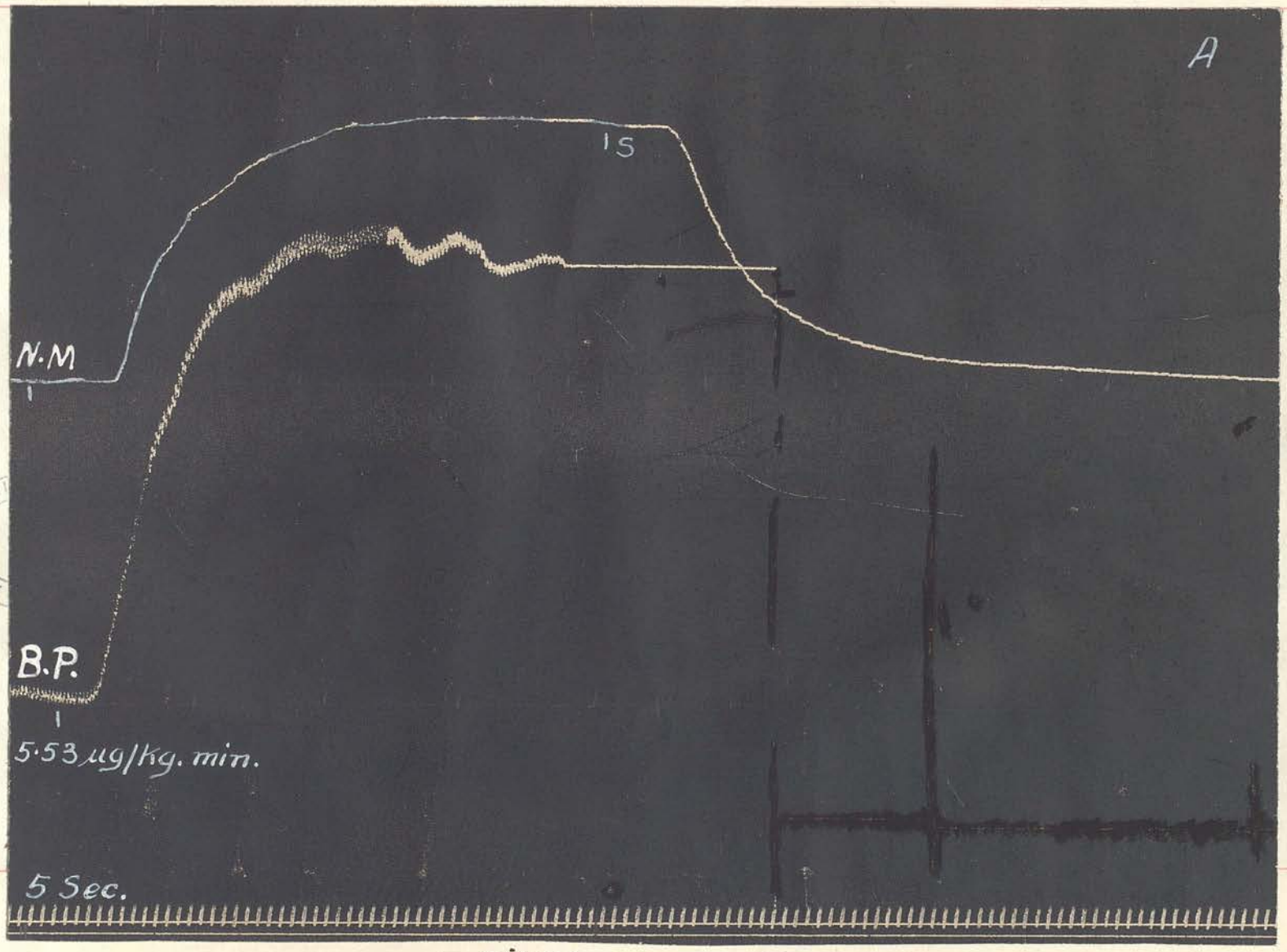


FIG.34a.

Fig. 34b. Responses of the n.m. and blood pressure of cat to continuous intravenous infusion of 5.53 µg/kg./min. adrenaline, when 37.64 mg/kg/min. administration of ascorbic acid was started about 6 min. before and continued throughout the course of the response.

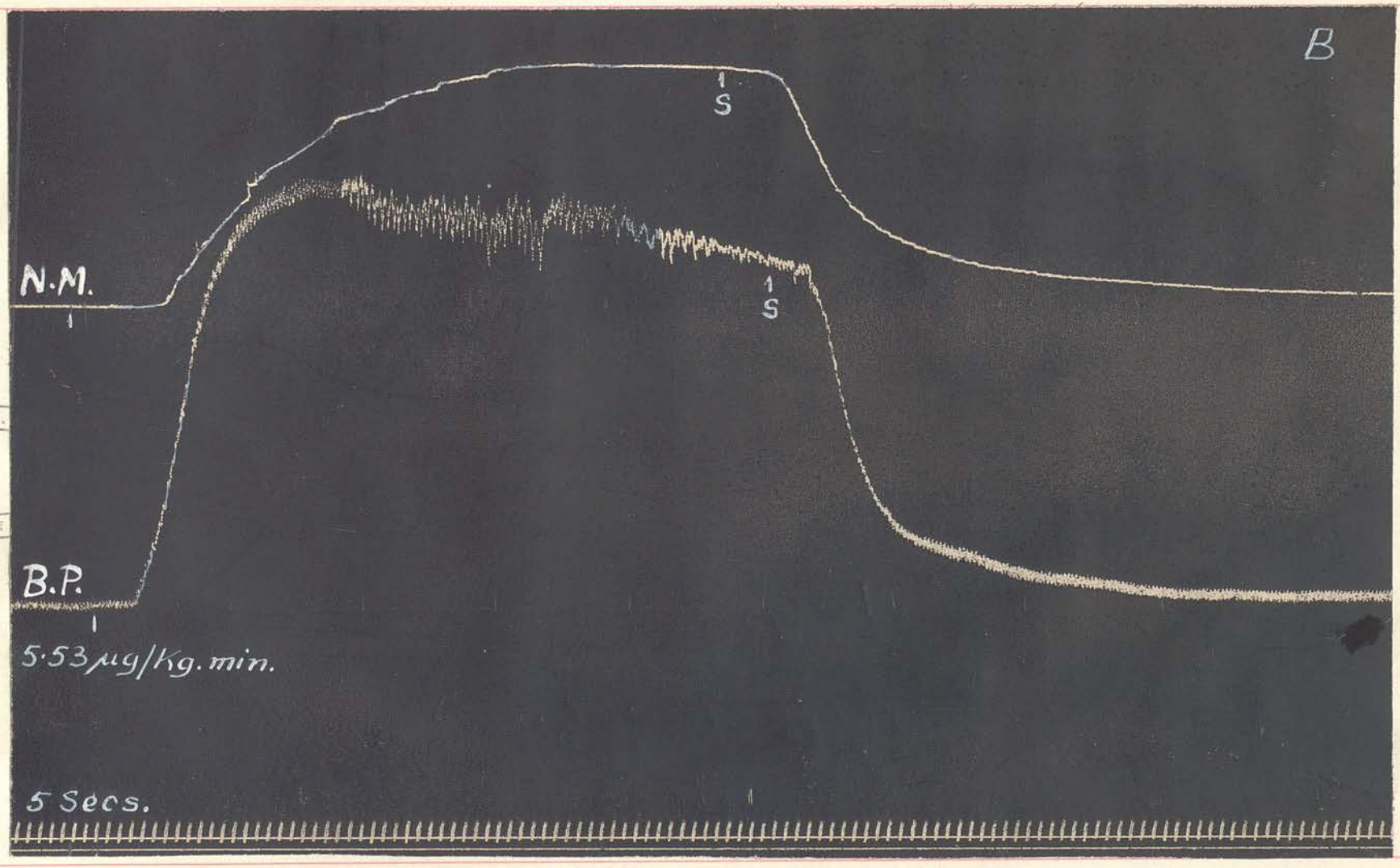


FIG.34b.