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QUANTITATIVE STUDIES OF PROCAINE METABOLISM IN THE CAT

BY

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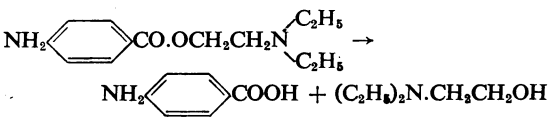
During the past few years there have been references to therapeutic uses of intravenously administered procaine, mostly to produce analgesia (Lundy, 1942; Gordon, 1943; McLachlin, 1945; Allen, 1945) and also to counteract cardiac arrhythmias (Burstein and Marangoni, 1940; Burstein, 1946).

Intravenous administration of a drug as toxic as procaine, whether by a single rapid injection or by a slow infusion, involves a consideration of the rates of dilution, diffusion, fixation, excretion, and destruction of the drug in the body. Eggleston and Hatcher (1916) showed that the toxicity of intravenous procaine increased directly with the rate of injection, and Macdonald and Israels (1932) and Hill and Macdonald (1935) demonstrated the same relation for depression of breathing. Eggleston and Hatcher (1916) also showed, by perfusion experiments in cats, that procaine is destroyed in the liver, but, using a biological test for the estimation of procaine concentrations, they observed no destruction of procaine by the blood in dogs. These results were confirmed by Dunlop (1935) in experiments on dogs, in which procaine and *p*-aminobenzoic acid blood levels were determined by a diazotization method, using β-naphthol as coupling reagent; after hepatectomy he still found some destruction of procaine which was attributed to an action by tissues other than the liver, though blood itself appeared to be inactive. Recently, however, Goldberg, Koster, and Warshaw (1943), Kisch, Koster, and Strauss (1943), and Hazard and Ravasse (1945) have adapted the Bratton and Marshall (1939) method for sulphonamides to the determination of procaine and *p*-aminobenzoic acid in blood and tissue fluids. They have shown that the plasma and serum of many species of animals, including man (in whom the effect is most powerful of all), can hydrolyse procaine to *p*-aminobenzoic acid and diethylaminoethanol. They ascribe this action to the presence of an enzyme, "procaine esterase," which is said to be distinct from cholinesterase, tropinesterase, and lipase.

Our work was designed to study procaine blood levels in cats after rapid intravenous injection, or slow infusion at various rates, with the special aim of determining the contributions made by diffusion, fixation, excretion, and destruction to the rapid removal of procaine from the blood. The parts played by the liver and kidney and the effects of physostigmine, neostigmine, methylene blue, and di*iso*propylfluorophosphonate on procaine metabolism have also been studied.

Chemical reactions involved in experiments

Procaine is hydrolysed as follows:—



p-Aminobenzoic acid may be acetylated, a reaction which occurs slowly in the liver and which is negligible during the perfusion experiments to be described. The amount of acetylation is considerable in the rabbit and in man, but is small in the cat (Ansbacher, 1944). *p*-Aminobenzoic acid may also be conjugated with glycine to form *p*-aminohippuric acid. This reaction is very slight in man and in the cat (Beyer, Mattis, Patch, and Russo, 1945).

METHODS

Cats were anaesthetized with chloralose (80 mg./kg. body weight). Arterial blood pressure was recorded by a cannula in one common carotid artery. One femoral artery was cannulated for collection of arterial blood samples. The injections or infusions of procaine were given into the femoral vein of the opposite side. Infusions were kept at a constant rate

by the use of a Marriotte bottle and a drip bulb, into which was incorporated a platinum loop connected to a Gaddum drop recorder to give a graphic record on the smoked drum. In this way changes in rate of flow were soon noticed and corrected. Procaine hydrochloride was made up to 0.3–0.5 per cent (w/v) in 0.9 per cent (w/v) NaCl solution. The rate of administration is expressed as mg. of procaine hydrochloride/kg. body wt./min.

Femoral artery blood samples were collected into a mixture of sodium fluoride and potassium oxalate to give a final concentration of $M/2$ NaF and $M/20$ $C_2O_4K_2.H_2O$. The blood-oxalate-fluoride mixture was then placed in a refrigerator at $-5°$ C. It had previously been shown that at room temperature less than 5 per cent of procaine was destroyed in 1 hour in contact with this mixture, and on freezing the loss was undetectable. At the end of an experiment 2 c.c. of each blood-oxalate-fluoride mixture were added to 8 c.c. of 12.5 per cent (w/v) trichloroacetic acid in order to precipitate proteins and provide an acid medium in which procaine is stable.

Procaine and *p*-aminobenzoic acid concentrations in blood and urine were determined by a modification of the method described by Kisch and Strauss (1943). The procaine was extracted in narrow tubes into chloroform (instead of ether) and re-extracted into 0.6 *N*.HCl in which the procaine concentration was then determined by the method of Bratton and Marshall (1939) as used for sulphonamides. Similar determinations were made on the blood filtrate, directly and after boiling for 1 hour with 6*N*.HCl; these enabled the concentrations of free and acetylated *p*-aminobenzoic acid to be calculated.

The effect of the liver on procaine destruction was studied by noting the change in blood procaine concentration produced by excluding the liver from the circulation, in a manner similar to that described by Smyth (1947) in his studies on acetate metabolism. The whole of the portal area was excluded from the circulation and the hepatic artery was isolated but left patent. It was found that when a constant rate of procaine infusion had been established for about 15 min. the blood level had become constant. The hepatic artery was then clipped so as to cut off nearly all the remaining blood flow through the liver, and the infusion was continued at the same rate.

Results

Continuous infusion of procaine

Procaine infusions have been given at different rates for periods of up to 30 min. At the beginning of the infusions there was frequently a 10–15 mm. Hg rise in blood pressure, but the rates of flow studied did not subsequently reduce blood pressure or depress breathing except with rates exceeding 2.0 mg./kg. body wt./min.

In Fig. 1 the effect of an infusion of 0.8 mg./kg./min. of procaine for 18 min. is shown. The blood

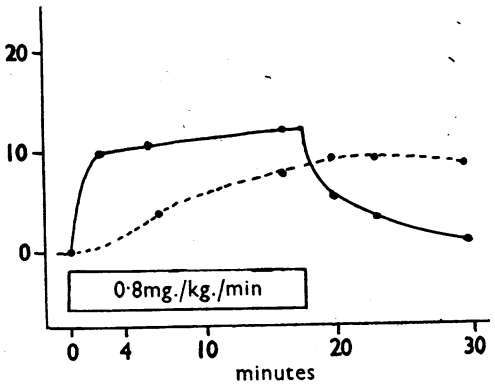


FIG. 1.—Curves of procaine and *p*-aminobenzoic acid blood concentrations during and after an infusion of 0.8 mg. procaine/kg./min. in a cat. Ordinates: concentration of procaine or *p*-aminobenzoic acid in mg./l. Abscissae: time in min. ●——● = procaine; o- - - - -o = *p*-aminobenzoic acid; ▭ = period of infusion.

level of procaine rose within 3 min. to 9.5 mg./l. and subsequently to 12 mg./l. after 16 min. Within 2 min. of stopping the infusion the blood level had fallen to 5 mg./l. and 12 min. after the end of the infusion it was 2 mg./l. The level of *p*-aminobenzoic acid rose during the course of the infusion to 7.5 mg./l. and after the end of the infusion a further slight rise to 9 mg./l. was noted; 12 min. after the end of the infusion it was still 8.5 mg./l.

In Fig. 2, 2.5 mg./kg./min. of procaine was infused for 12.5 min. At 2.5 min. the blood level was 35 mg./l., at 5 min. it was 55 mg./l., and at 12 min. it had risen to 66 mg./l. After the infusion was

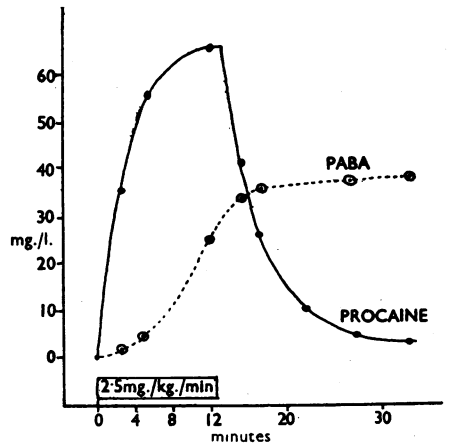


FIG. 2.—Ordinates and abscissae as in Fig. 1. Procaine infusion at 2.5 mg./kg./min. Procaine and *p*-aminobenzoic acid (PABA) blood levels during and after infusion.

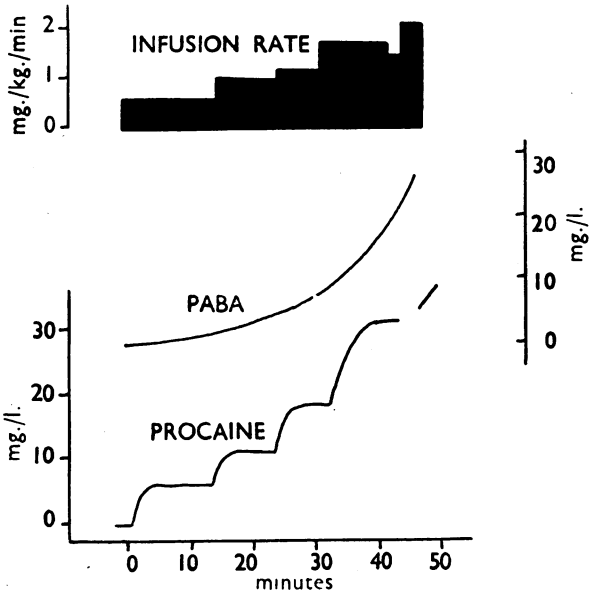


FIG. 3.—Curves of blood procaine and *p*-aminobenzoic acid concentrations at different rates of infusion of procaine. Upper part of record: rate and duration of infusion. Lower part of record: procaine and *p*-aminobenzoic acid (PABA) blood concentrations. Ordinates on left: procaine. Ordinates on right: *p*-aminobenzoic acid.

stopped the blood procaine level fell rapidly, and within 2 min. of cessation it was 40 mg./l., within 5 min. it was 25 mg./l., and subsequently it declined more slowly to 3 mg./l. by 20 min. after the infusion had ended.

The effects of successive increases in rates of infusion on blood procaine levels are shown in Fig. 3. At rates of 0.5–1.6 mg./kg./min. the blood curves flatten out by the end of the infusion. The infusion at 2.1 mg./kg./min. was not maintained long enough to reach a plateau level, owing to the development of respiratory failure. It was found that whenever the infusion had been tolerated for a sufficient period (10–30 min. depending on the rate of infusion) a constant blood procaine level was reached, the height of which was proportional to the rate of infusion. The relation between infusion rate and blood level of procaine is shown in Fig. 4, which summarizes the data set out in Fig. 3. The blood levels indicated in Fig. 4 are the equilibrium values.

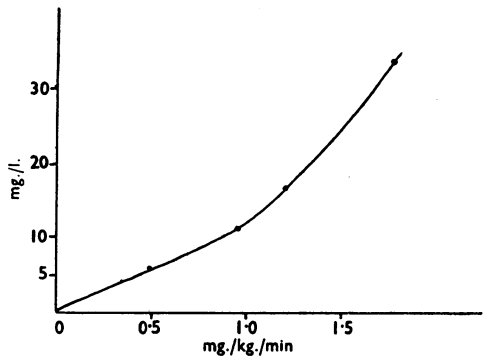


FIG. 4.—Relation of infusion rate to blood procaine concentration. Data from curves of Fig. 3. Ordinates: concentration of procaine in blood in mg./l. Abscissae: infusion rate of procaine in mg./kg./min.

Single injections of procaine

In one experiment single injections of procaine were given (each injection took 30 sec.) and blood levels were determined $\frac{1}{4}$ min., $1\frac{1}{4}$ min., $3\frac{1}{4}$ min., and $5\frac{1}{4}$ min. after completion of the injection. An interval of 30 min. was allowed between each injection so that blood procaine levels could return to zero before the next injection. Fig. 5 shows the initial high blood procaine concentrations and the subsequent very rapid decreases after injections of 5, 10, 20, and 30 mg. of procaine. The highest rate of fall is during the first minute after the end of the injection—e.g., after 30 mg. procaine the blood level was 77 mg./l. at $\frac{1}{4}$ min. and 22 mg./l. at $1\frac{1}{4}$ min. after the end of the injection.

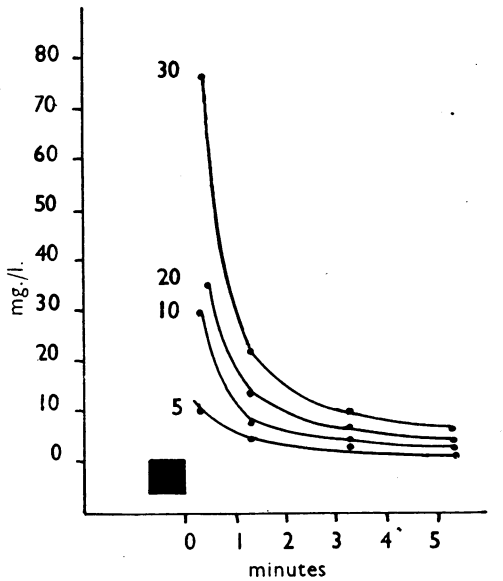


FIG. 5.—Blood procaine concentrations after single injections of procaine. In each curve the numeral at the top shows the dose in mg. of procaine injected. The black square indicates the period of injection ($\frac{1}{2}$ min.).

Interpretation of blood level changes after single injections of procaine

It will be seen in Fig. 5 that after a single injection of 30 mg. procaine in 30 sec. the blood procaine level fell from 77 mg./l. to 7.5 mg./l. within 5 min. of completion of the injection. Reference to Fig. 2 shows that after the termination of an infusion of procaine the blood level fell from 65 mg./l. to 25 mg./l. within the same period. The factors which produce this significant difference in rates of decay will now be considered.

After a single injection the fall in blood procaine level may be due to the following factors:—

1. Diffusion from the blood stream into the tissue fluids and cells.
2. Fixation by tissues.
3. Destruction in the blood stream, tissue fluids, and cells.
4. Excretion.

It will be shown later that urinary excretion is negligible, and there is no evidence for significant elimination in any other way, so that the difference between the decay curve after a single injection and that after a continuous infusion must be related to the other factors. After a single injection lasting ½ min. there must be a considerable element of diffusion from the blood into the tissues,

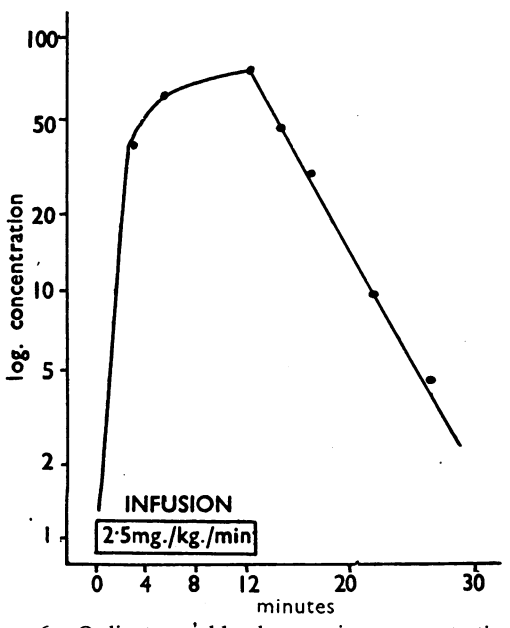


FIG. 6.—Ordinates: blood procaine concentrations (from Fig. 2) on logarithmic scale. Abscissae: time in min. Note the straight line decay in procaine concentrations.

whereas after the end of an infusion, when a plateau blood level has been reached, this factor would be negligible. Since the blood contains a higher concentration of the enzyme procaine esterase than any other tissue (Kisch, Koster, and Strauss, 1943) this blood enzyme would seem to be the most important factor in the fall in blood procaine level after the end of an infusion. If this were so there should be an exponential decay curve typical of such enzymatic processes, and Fig. 6 shows that when the decay curve from Fig. 2 is replotted with blood procaine concentrations on a logarithmic scale a straight line relationship is revealed. This observation, of course, does not locate the site of enzymatic destruction, whether in blood or tissues, but it does help to distinguish the factor of diffusion from that of destruction in the following way: If it be assumed that 5 min. after a single ½ min. injection of procaine diffusion from the blood has virtually ceased, and further that even diffusion into the whole body water (intracellular and extracellular) has occurred, then it is possible to calculate what proportion of the decay curve is due to diffusion and what to enzymatic destruction.

In the experiment recorded in Fig. 5 the cat weighed 2.2 kg., and according to Gregersen (1941) the blood volume would therefore be 250 c.c., the extracellular fluid volume 550 c.c., and the total body water 1,500 c.c. If 30 mg. procaine be evenly distributed throughout the total body water the blood level would be 20 mg./l. and if diffusion were incomplete a higher value would be found. The actual blood level 5 min. after completion of the injection was 7.0 mg./l. Similarly, the procaine concentrations to be expected after 20, 10, and 5 mg. injections would be 13.5, 6.8, and 3.4 mg./l. respectively; the actual blood levels were 4.5, 2.0, and 0.9 mg./l. It is therefore evident that in each case about two-thirds of the injected procaine has been destroyed or fixed in the tissues in 5 min. After injection of 10 mg. of procaine the *p*-aminobenzoic acid level (2.5 mg./l.) suggested that the disappearance of this fraction of procaine was due almost entirely to hydrolysis and not to tissue fixation. From the data given by the decay curve in Fig. 2 the reduction in blood procaine levels due to enzyme action can be estimated (see Appendix) and the reduction due to diffusion can be calculated by difference from the values given in Fig. 5. In Fig. 7 the continuous line shows the curve of enzymatic breakdown that would be expected if diffusion were instantaneous, and the dotted line the fall in blood level produced by diffusion alone.

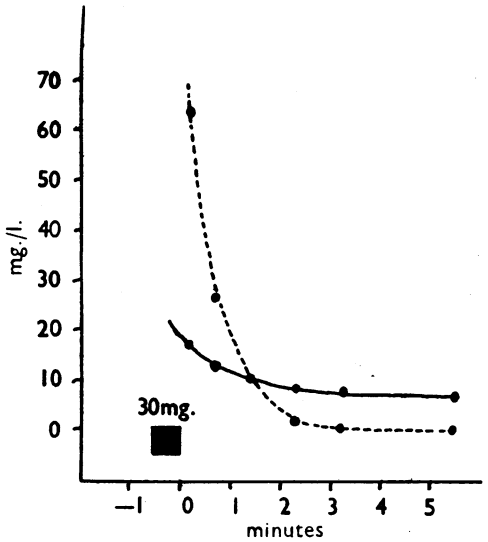


FIG. 7.—Curves showing contributions of diffusion (o----o) and enzymatic destruction (●——●) to fall in blood procaine concentrations after injection of 30 mg. procaine in ½ min. The curves have been obtained by calculations described in the text and the Appendix, and should be compared with that after 30 mg. procaine in Fig. 5.

The kidney and procaine blood levels

The possibility that the kidney might destroy or excrete infused procaine quickly enough to influence blood levels was studied:

1. By determining blood procaine levels before and after ligation of the renal pedicle.

2. By measuring procaine excretion in the urine.

1. Fig. 8 shows the results of an experiment in which an infusion of procaine at a rate of 1.2 mg./kg./min. was given for 30 min. In the first infusion a peak procaine level of 22 mg./l. was recorded; the *p*-aminobenzoic acid level rose steadily and remained at 20 mg./l. when the procaine level had fallen to 2.5 mg./l. after the end of the infusion. The renal pedicles were then ligatured tightly to cut off all blood flow through the kidney, 80 min. after the end of the first infusion. The infusion was then restarted at the same rate as before and continued for 30 min. The peak blood procaine level was 24 mg./l. at the end of the infusion period. The *p*-aminobenzoic acid level which was 17.5 mg./l. at the beginning of the second infusion rose more steeply than on the first occasion and finally reached a value of 50 mg./l. at 30 min. after the end of the infusion.

The difference between the peak procaine values of 22 mg./l. during the first infusion and 24 mg./l. during the second infusion (after tying the renal pedicles) is very small and suggests that procaine is neither destroyed nor excreted to any significant degree by the kidney. The steeper rise in *p*-aminobenzoic acid levels after ligature of the renal pedicle suggests a significant excretion of this substance by the kidney.

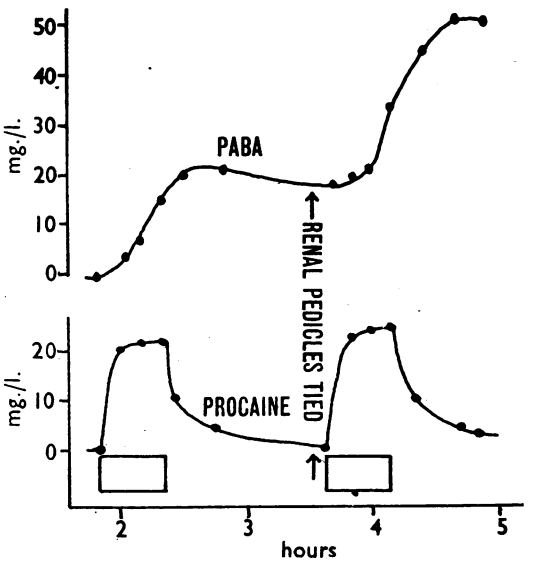


FIG. 8.—Effect of tying both renal pedicles on procaine and *p*-aminobenzoic acid blood concentration after infusions of procaine (1.2 mg./kg./min.).

2. Urinary excretion of procaine and *p*-aminobenzoic acid was studied in both cats and man. In Fig. 9 (b) the excretion of procaine and *p*-aminobenzoic acid in the urine of a cat is recorded for 2½ hr. during and after an infusion of procaine at a rate of 0.8 mg./kg./min. for 25 min. The blood level was steady at 21 mg./l. 2.1 per cent of the injected procaine was excreted as such during the infusion and in the subsequent 2 hr. a further 1.4 per cent was recovered. This rate of excretion could play no significant part in the fall in blood level after the infusion ended. The excretion of *p*-aminobenzoic acid was much greater.

In Fig. 9 (a) the excretion of procaine and *p*-aminobenzoic acid in human urine is recorded during and after an infusion of 2 g. of procaine in 70 min., urine being collected for a total period of 5¼ hr. Only 0.3 per cent of the injected procaine appeared in the urine during this period. The smaller proportion of procaine found in human urine may be related to the finding of Kisch *et al.*

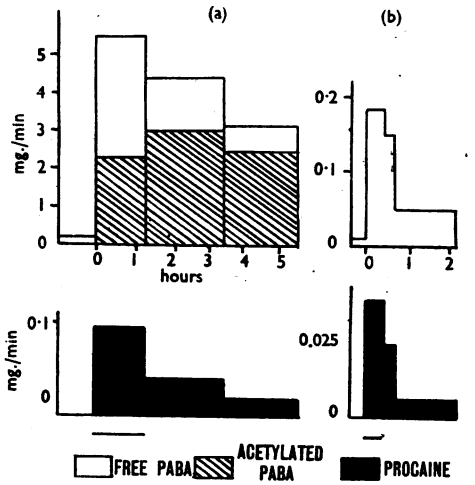


FIG. 9.—Urinary excretion of procaine, *p*-aminobenzoic acid (PABA), and acetylated PABA after procaine infusions in (*a*) a normal human subject and (*b*) a cat. Ordinates: amounts of the above compounds excreted in mg./min. Abscissae: time in hr. The black horizontal lines indicate the periods of infusion: (*a*) 2 g. procaine in 70 min.; (*b*) 0.8 mg./kg./min. for 25 min.

(1943) that the procaine esterase activity of human blood is greater than that of cat's blood. In human urine it will be noticed that a large proportion of *p*-aminobenzoic acid is acetylated; in cat's urine the acetylated fraction is negligible.*

The liver and procaine blood levels

The part played by the liver in the destruction of procaine was investigated by recording the effect of excluding the liver from the circulation on blood procaine levels during an infusion in an eviscerated cat.

In Fig. 10 a procaine infusion of 0.65 mg./kg./min. produced a plateau blood level of 14 mg./l. Twenty min. after the beginning of the infusion the hepatic artery was clipped and the infusion continued at the same rate. The blood procaine level rose rapidly to 24 mg./l. and after 20 min. of arterial occlusion the level was 25 mg./l.; *p*-aminobenzoic acid blood levels rose steadily throughout the whole infusion period and there was no significant change in the slope of the curve during the period of hepatic artery occlusion.

It was thought possible that the liver might be concerned with the synthesis and liberation into the blood stream of procaine esterase, as has been claimed for cholinesterase by Brauer and Root (1946). The increased toxicity of procaine after liver damage noted by Ellinger and Hof (1929) might therefore be due to a reduction in blood procaine esterase. To test this hypothesis liver damage was produced by intraperitoneal injection of carbon tetrachloride into rats and rabbits. Each rat was given 0.075 c.c. of CCl_4 on five occasions at 2-day intervals. The rabbits were given 0.4 c.c./kg. on the 1st day and 0.2 c.c./kg. on the 3rd and 5th days. Serum procaine esterase determinations were made before and at intervals during the period of CCl_4 administration, using the method described by Kisch and Strauss (1943). In rats the normal serum procaine esterase values were too small to give useful information, and the rabbits, in which high procaine esterase concentrations were found, showed no reduction with liver damage.

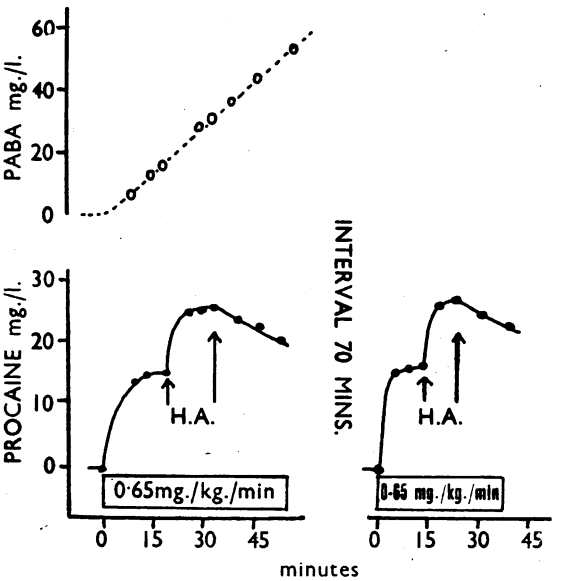


FIG. 10.—The effect of excluding the liver from the circulation during infusions of procaine. Upper scale: *p*-aminobenzoic blood values. Lower scale: procaine blood values. The experiment was done as described in the text in an eviscerated cat with the hepatic artery isolated and left patent. Between the arrows the hepatic artery was clipped.

Effects of physostigmine, neostigmine, methylene blue, and diisopropylfluorophosphonate on blood procaine and procaine esterase

Kisch (1943) reported a powerful inhibition of procaine esterase *in vitro* by physostigmine, neo-

* Since this paper was submitted for publication Krebs, Sykes, and Bartley (1947) have found that in many animals, including cats but not man, there are enzymes which deacetylate N_4-acetylsulphonamides and which presumably could deacetylate *p*-acetaminobenzoic acid. The presence of such enzymes probably accounts for the very small amounts of *p*-acetaminobenzoic acid found in the blood and urine of cats after procaine administration.

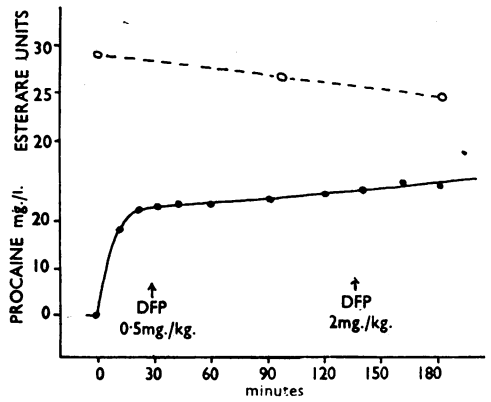


FIG. 11.—The effect of di*iso*propylfluorophosphonate (DFP) on blood procaine and procaine esterase concentrations during a constant infusion of procaine at 1.0 mg./kg./min. The procaine esterase concentration is recorded above in arbitrary units. The procaine blood levels are recorded below. At the arrows DFP was injected.

stigmine, and methylene blue. Doses of 1 mg. physostigmine, 0.75 mg. neostigmine, and 10–50 mg. methylene blue had no effect on blood procaine levels during infusions into cats. Di*iso*propylfluorophosphonate (DFP), in doses of 0.5 and 2 mg./kg., as shown in Fig. 11 also produced no significant change in blood procaine level during an infusion of procaine at 1 mg./kg./min. The slight fall in serum procaine esterase activity and the slow small rise in blood procaine level noted in this record are probably due to the effects of repeated withdrawals of blood for determination of procaine concentrations.

DISCUSSION

The administration of intravenous infusions of procaine, as described by Lundy (1942), Gordon (1943) McLachlin (1945), and Allen (1945, 1946), must be closely associated with the rate of inactivation of this compound in the body. Rates of 10–50 mg. procaine/min. can be administered to human subjects for several hours with no evidence of cumulative action, and rates of up to 150 mg. of procaine/min. have been given for short periods, so that the processes of detoxication are extraordinarily rapid and efficient; this is also shown by the speed of recovery from the effects of intravenous procaine.

The rates of infusion of procaine administered to cats correspond very closely to those given in man. In cats, as observed by Hill and Macdonald (1935) and confirmed by ourselves, rates in excess of 2 mg./kg./min. are liable to produce respiratory failure; this would be equivalent to 12 c.c. of 1 per cent procaine/min. in a 60 kg. man. It might however be expected that man would tolerate more than this by virtue of the greater procaine esterase activity of human blood, which is about 3 times that of cat's blood (Kisch *et al.*, 1943).

It is clear from the results recorded in Fig. 8 and Fig. 9 that the kidney plays an insignificant part in the destruction or excretion of procaine, and the fact that in man less than 0.5 per cent of the injected procaine appears unchanged in the urine within a few hours shows that urinary excretion is of no importance.

Since the marked tolerance to intravenous procaine is not due to very rapid excretion, it may be assumed that the drug is speedily inactivated in the body. Destruction of procaine by the liver was shown by Eggleston and Hatcher (1916) and by Dunlop (1935) and was postulated by Ellinger and Hof (1929), and destruction by blood *in vitro* has been demonstrated by Kisch *et al.* (1943) and Hazard and Ravasse (1945). The question therefore arises as to how much the blood and liver respectively are responsible for the inactivation of procaine. Our experiments on eviscerated animals give some idea of the proportion of procaine which may be destroyed by the liver. The rate of procaine destruction bears an inverse relationship to the blood level (plateau level); the rate after exclusion of the liver is thus 14/24ths of the original rate, the liver having been responsible for the remainder of the destruction—viz., about 40 per cent of the total procaine catabolism.

It was thought possible that the liver might secrete procaine esterase into the blood stream in a manner similar to that described for cholinesterase by Brauer and Root (1946), who showed that liver damage produced by carbon tetrachloride reduced the plasma cholinesterase level in rats, though subsequently Ellis, Sanders, and Bodansky (1947) have found that this does not occur in rabbits. Our experiments on serum procaine esterase levels in rats showed such low concentrations of this enzyme that the effects of liver damage could not be assessed. In rabbits, in which procaine esterase determinations were easily performed, liver damage caused no reduction in serum concentrations of this enzyme. From this it is concluded that procaine esterase is not released from the liver into the general circulation in the rabbit.

Kisch *et al.* (1943) found that physostigmine, neostigmine, and methylene blue all inhibited procaine esterase *in vitro*. Neither these substances nor di*iso*propylfluorophosphonate had any effect in full doses on the procaine blood levels in cats.

With slow infusions, once the initial diffusion

has occurred, the maintenance of a steady blood level is largely if not entirely the result of enzymatic destruction, and it is interesting to note that plateau blood levels are reached with all rates of infusion below those which depress respiration. In a cat weighing 3 kg., 5 mg. of procaine can be inactivated per min.; in a man weighing 60 kg. this would correspond to 100 mg./min. which has often been given with apparent safety.

There seems to be little doubt that the destruction of procaine is in fact hydrolysis with the formation of *p*-aminobenzoic acid and diethylaminoethanol, the former of these two compounds having been frequently detected and estimated in blood and urine in the course of these experiments. The continued presence of fairly high *p*-aminobenzoic acid blood concentrations after the blood procaine levels have fallen almost to zero suggests that procaine which is bound to tissues immediately after diffusion is either slowly hydrolysed by such tissues or is liberated slowly from these tissues into the blood stream where hydrolysis takes place.

We are very grateful to Mr. W. F. Floyd for helpful criticisms of this work, and to Mr. F. J. Haydon for technical assistance.

SUMMARY

1. Blood procaine and *p*-aminobenzoic acid blood levels were determined in chloralosed cats after procaine infusions at rates of 0.5–2.5 mg./kg. body wt./min., and after short single injections of 5–30 mg. of procaine.

2. With infusion rates of 0.5–2.5 mg./kg. body wt./min., maximum blood procaine levels of 6–66 mg./l. were recorded, and where the infusion was tolerated for at least 10 min. constant blood levels were always attained. Simultaneous *p*-aminobenzoic acid blood levels suggested rapid and extensive hydrolysis of procaine.

3. Blood procaine decay curves were determined in six cats and found to be logarithmic.

4. After short single injections of procaine the rapid fall in blood level during the first two minutes after the injection is due almost entirely to diffusion from the blood into the tissues.

5. The kidney plays no significant part in the reduction of procaine blood levels during and after procaine infusions, but excretion of *p*-aminobenzoic acid is rapid and considerable.

6. The liver is responsible for destruction of up to 40 per cent of the procaine metabolized in the cat.

7. There is no conclusive evidence that the liver "secretes" procaine esterase into the blood stream (rabbit).

8. Physostigmine, neostigmine, methylene blue, and DFP do not influence blood procaine levels during procaine infusion.

APPENDIX

(Written with the help of Mr. W. F. Floyd of the Department of Physiology, Middlesex Hospital Medical School.)

Note on the calculation of enzymatic hydrolysis after rapid injection

When the type of substrate concentration–velocity curve for the enzyme is known the rate of enzymatic hydrolysis can be calculated from the mean rate of breakdown over the whole period of observation. After a single rapid injection the blood concentration is much higher than the tissue concentration during the first few minutes. Thus the assumption made here that the procaine is hydrolysed only in the blood will probably not involve a large error.

When the concentration of procaine in the blood

1	2	3	4	5
Time intervals min.	C_t(mg./l.)	$C_tKm\delta t$(mg./l.)	$\frac{W_0}{a} - \sum_0^\tau C_tKm\delta t$	Diffusion
0–0.25	95	2.66	17.34	77.66
0.25–0.75	64	3.55	13.79	50.21
0.75–1.25	36	2.00	11.79	24.21
1.25–1.75	26	1.45	10.34	14.66
1.75–2.25	11.8	0.65	9.69	2.11
2.25–2.75	9.6	0.56	9.13	0.47
2.75–3.25	8.4	0.47	8.66	−0.26
3.25–3.75	8.0	0.44	8.22	−0.22
3.75–4.25	7.6	0.42	7.80	−0.20
4.25–4.75	7.3	0.40	7.40	−0.10
4.75–5.25	7.0	0.39	7.01	0

is C_t, the concentration hydrolysed in the small time interval δt (C_t being the mid-interval concentration) is obtained by multiplying C by a coefficient proportional to the mean rate of breakdown occurring in unit time (i.e., the product Km) and by δt.

Hence the total concentration hydrolysed in the time interval $O \rightarrow T$ is the sum of the concentrations hydrolysed in the successive time intervals δt. This is equal to the difference between the total amount injected (W_o) and that still unhydrolysed at time T (W_T) divided by the fluid volume (a) in which they are dispersed—

$$\text{i.e.,} \quad \sum_{t=o}^{T} C_t Km\, \delta t = (W_o - W_T)/a.$$

(Note: the parameter K in the product Km is the velocity constant of the enzyme reaction ; m is proportional to the enzyme concentration in the blood and for any one animal is a constant.)

An example of the working for an injection of 30 mg. of procaine into a 2.2 kg. cat with a total body water of 1.5 l. is given in the Table.

In order to calculate the entries for column 3 of the Table it is necessary to determine the value of Km as follows:—

$$\sum_{o}^{5.25} C_t = 233 \text{ (i.e., sum of total values of } C_t).$$

$W_o = 30$ mg. $W_T = 10.5$ mg. $a = 1.5$ l.
$\delta t = 0.5$ minutes.

$$Km = \frac{W_o - W_T}{a.\delta t.\ \sum_{o}^{T} C_t} = \frac{30 - 10.5}{1.5 \times 0.5 \times 233} = 0.1114$$

In Fig. 7 the continuous curve is that given by column (4) and the dotted curve by column (5).

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