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EXPERIMENTS ON THE FATE OF HISTAMINE AND ACETYLCHOLINE AFTER THEIR INJECTION INTO THE CEREBRAL VENTRICLES

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Drugs injected into the lateral ventricles of the brain are known to produce profound and long-lasting effects, but it is not known how long they remain in the ventricular system and whether the long-lasting effects depend upon their persistence in the ventricular spaces.

In the experiments to be described an attempt was made to determine the fate of histamine and acetylcholine injected into the lateral cerebral ventricles of cats and dogs by assaying the amounts recovered at different times after the injection in washings of the cisterna magna and the ventricles, or in the epicerebral or spinal c.s.f. Further, the histamine absorbed into the blood stream was estimated by its effect in producing acid gastric secretion, and some observations were made on circulatory and respiratory effects produced by the intraventricular injections.

METHODS

Cats (2–3·5 kg) and dogs (5–17 kg) were anaesthetized with either pentobarbitone sodium (36 mg/kg) intraperitoneally or chloralose (8 ml. of a 1% solution/kg) intravenously.

Cannulation of the cat's ventricle. The head of the cat was securely supported by a head holder and raised about 10 cm above the plane of the operation table. The Collison cannula for intraventricular injection described by Feldberg & Sherwood (1953) was used, modified so that there was only one side hole at about 1 mm from its occluded tip. A track was created in the brain before insertion of the cannula, by the use of a 'pilot-guide' (Fig. 1*a*). It consists of a metal cylinder tunnelled centrally throughout, and carrying a screw thread on its lower narrower part, which serves to screw it into the skull, and a metal shaft, which is passed through the hole of the cylinder and through the brain until the tip reaches the ventricle.

Cannulation of the dog's ventricle. The method was the same, in principle, as in cats. However, on account of the greater variation in the size of the dog's brain the length of the cannula had to be varied through a wider range and therefore the cannula had to be frequently inserted and taken out before its tip rested in the ventricle. To prevent damage by this repeated procedure to the thread cut in the skull, a metal sleeve was screwed into the skull and the cannula screwed into the

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sleeve. The sleeve, which is illustrated in Fig. 1*b*, was introduced by A. C. Palmer (unpublished experiments) for cannulation of the cerebral ventricle of the sheep. The thread on the outer surface of the sleeve which serves to screw it into the skull was $\frac{1}{4} \times 40$ B.S.F., while the thread on its inner surface which takes the thread of the cannula was the usual 3 B.A. thread.

Intraventricular injections. Histamine acid phosphate, acetylcholine chloride and eserine sulphate were slowly injected in a volume of 0·25 ml. of magnesium-free Tyrode solution, followed after about 20 sec by another slow injection of 0·25 ml. of Tyrode solution in order to wash in the drug. All values of histamine refer to the base and those of acetylcholine and eserine to the salt.

Collection of c.s.f. from the cisterna magna. The cannula used was a 3 cm long 20 S.W.G. hypodermic needle devoid of its butt and entirely surrounded by a closely fitting steel tube, except for the terminal 3 mm of the sharp end to be inserted into the cisterna. The other end was attached to a small length of polythene tubing and kept closed, during the times no fluid was collected, by a small glass stopper. In order to keep the cannula in position a pointed iron rod with a hole in its centre was first passed through the retracted muscles of the neck so that the hole faced the

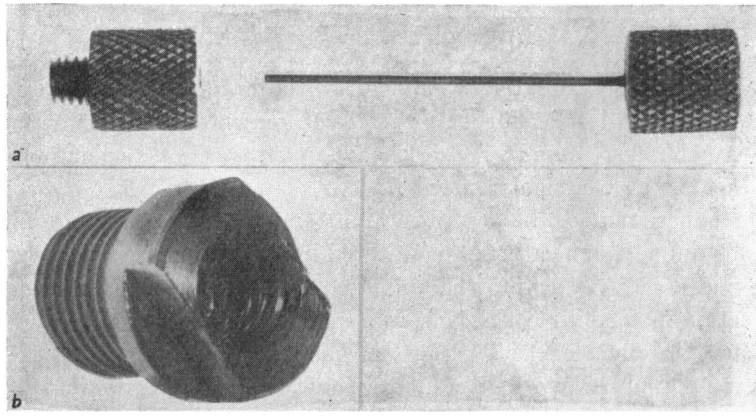

Fig. 1. Pilot guide (*a*) and sleeve (*b*) used for cannulation of the lateral cerebral ventricle.

middle of the exposed atlanto-occipital membrane. The cannula was then passed through the hole of the iron rod and the membrane into the cisterna. On removal of the glass stopper, the cerebrospinal fluid started to flow out. In cats between 0·5 and 2·4 ml., and in dogs 2–3 ml. c.s.f. were collected before the flow stopped. To restart it and to wash out the ventricular spaces, Tyrode solution in amounts of 1 ml. in cats and 2 ml. in dogs was injected intraventricularly 5–6 times at about 2 min intervals. Each injection led to an outflow a little in excess of the injected volume, which was collected as a separate sample.

In experiments using histamine the samples were assayed for histamine on the atropinized guinea-pig ileum; in those using acetylcholine, the assay was carried out on eserinized frog rectus muscle.

Collection of c.s.f. by lumbar puncture. Lumbar puncture was made by a 20 S.W.G. needle. About 0·3 ml. of c.s.f. could be collected in cats and about 2 ml. in dogs before the flow stopped. It could be restarted by intraventricular injections of 1–2 ml. of Tyrode solution. The butt of the needle could be closed by 'Plasticine' when samples were not being collected.

Collection of c.s.f. from epicerebral subarachnoid space. The ventricular cannula was on the right side and collection was effected through a hole bored in the skull at a corresponding point on the left side. On incision of the dura successive samples of 0·1–0·2 ml. were obtained.

Collection of samples of gastric secretion and estimation of their free HCl content. The procedure was that described by Edkins (1906). Cats were starved for 24 hr and anaesthetized by intraperitoneal pentobarbitone sodium. The abdomen was opened by a small median incision. The duodenum was tied off about 2 cm from the pylorus and a polythene tube was inserted into the stomach through a small cut on the antimesenteric border below the pyloro-duodenal junction. The stomach was washed out several times with 10 ml. warm saline solution introduced through the polythene tube and removed with a pipette, and then filled with 10 ml. of warm saline solution. The free end of the polythene tube was closed by means of a clamp and the solution allowed to remain in the stomach for 20 min. It was then removed and replaced with another 10 ml. of saline solution. This procedure was repeated every 20 min and the 20 min samples were titrated for their free HCl content against 0·01 N-NaOH using Töpfer's reagent as indicator. When the acid gastric secretion obtained on intraventricular injection was compared with that produced by slow intravenous infusion, the continuous slow injector (C. F. Palmer Ltd.) was used, and the histamine solution was infused through the femoral vein.

RESULTS

Circulatory and respiratory effects

In cats the intraventricular injection of 500 μg of either histamine or acetylcholine produced pressor and depressor effects. Typical instances are illustrated in Figs. 2 and 3. The effects occurred also after section of the vagi in the neck. Intraventricular injections of Tyrode solution were ineffective. When histamine produced a pressor effect it was always small and gradual as shown in Fig. 2A, but with acetylcholine the rise was often sharp and large, as shown in Fig. 3B. The depressor effect of histamine was also usually less pronounced and more transient than that of acetylcholine; it was sometimes followed by slow rhythmic variations in the arterial blood pressure. In addition, histamine produced tachycardia lasting for a few minutes, the heart rate increasing by up to 90 beats/min. When acetylcholine was injected together with 100 μg of eserine the depressor effect was more prolonged; it did not occur after intravenous atropine.

In dogs the intraventricular injection of 500 μg of histamine produced an initial transient gradual rise in arterial blood pressure often followed by slow rhythmic changes in blood pressure. There was no cardio-acceleration but respiration was sometimes stimulated. An intraventricular injection of 500 μg acetylcholine caused a small prolonged rise in arterial blood pressure and stimulation of respiration. A similar effect was obtained with eserine. If respiration was irregular, it became not only more frequent, but also regular. This is shown in Fig. 4.

The difference between the effects in cats and dogs is probably mainly due to the fact that in cats a small fraction of the injected histamine or acetylcholine is pushed into the subarachnoidal space, from where it would be quickly absorbed, whereas in dogs, with the much larger ventricular spaces, this would not occur. The finding that the depressor action in cats occurred usually after the second injection of 0·25 ml. Tyrode and that the depressor

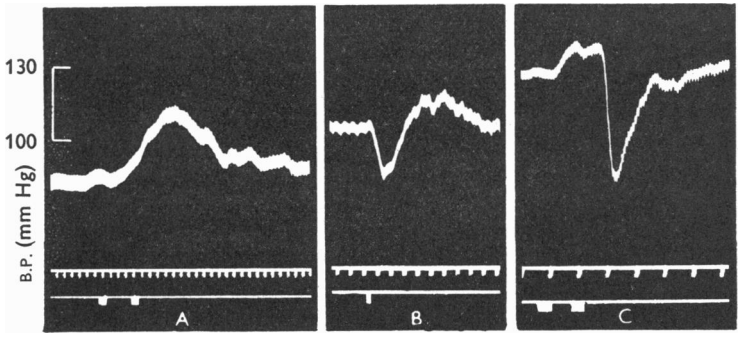

Fig. 2. Arterial blood pressure records from three cats. Effects of intraventricular injection of 500 μg of histamine. The two marks on the bottom line in A and C indicate the time of injection and of subsequent washing in of 0·25 ml. Tyrode solution; the single mark B indicates the end of washing in. In A and B, time marker 10 sec; in C, 30 sec.

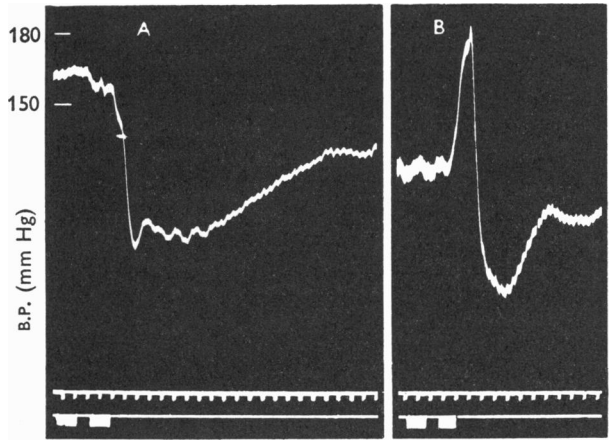

Fig. 3. Arterial blood pressure from two cats. Effects of intraventricular injections of 500 μg ACh. The two marks on the bottom line indicate the time of injection and of subsequent washing in of 0·25 ml. Tyrode solution. Time marker, 10 sec.

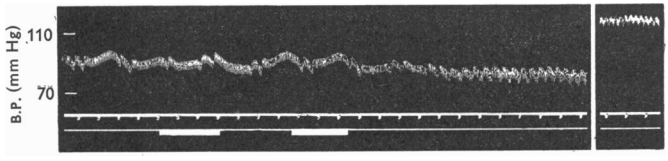

Fig. 4. Dog's arterial blood pressure recorded on a quick-moving drum to show individual heart beats and rate of respiration. Effect of intraventricular injection of 100 μg eserine. The second block 4 min after injection. The two marks on the bottom line indicate time of injection and of subsequent washing in with 0·25 ml. Tyrode solution. Time marker, 10 sec.

effect of acetylcholine did not occur after atropine would thus be easily accounted for. Since small doses of histamine injected intravenously increase the heart rate, the cardio-acceleration seen after intraventricular injection of histamine could also be an effect of absorbed histamine. On the other hand, the pressor effects and the stimulation of respiration are central in origin and so, probably, are the rhythmic changes in arterial blood pressure. A similar centrally produced blood-pressure effect has recently been described by Trendelenburg (1957) on injection of a small dose of histamine (10 μg) into the cerebral lateral ventricle of the anaesthetized cat.

Recovery in cats of injected histamine and acetylcholine from the cisterna magna

Cerebrospinal fluid collected from the cisterna had no effect on the atropinized guinea-pig's ileum but caused small contractions on the non-atropinized preparation when added in amounts of 0·1–0·4 ml. to the 15 ml. bath. The effect might be due to choline which is known to be present in small amounts in cerebrospinal fluid. The cerebrospinal fluid did not inactivate histamine as shown in experiments in which histamine was incubated with cerebrospinal fluid for 1 hr at 37° C.

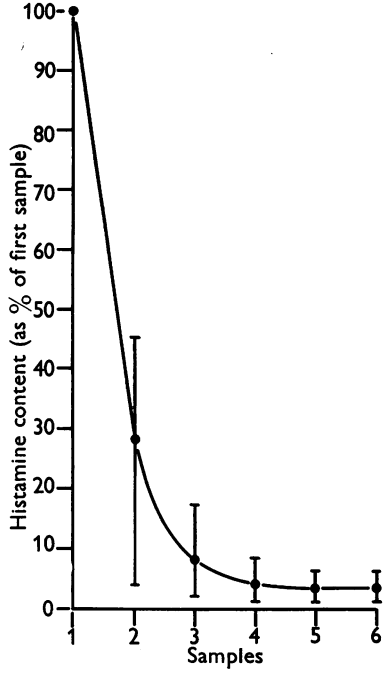

Fig. 5. Histamine recovery after intraventricular injection of 500 μg histamine in six successive samples collected from the cisterna magna in cats. Collection started 3 min after injection. The black dots give the mean values obtained in eight cats, the vertical lines the scatter of values.

Histamine. When 500 μg histamine was injected intraventricularly and 3 min later the collection of fluid from the cisterna was begun, between 40 and 77 % (mean 63 %) of the injected histamine was recovered. The first sample of undiluted c.s.f. collected from the cisternal cannula varied between 0·7 and 1·9 ml. and contained 27–56 % of the injected histamine. The histamine concentration in this sample varied between 1:3,000 and 1:11,000. The histamine content in the subsequent samples, each one collected during and after an intraventricular injection of 1 ml. of Tyrode solution to wash out the ventricular spaces, fell off steeply. This is illustrated in Fig. 5. It was not possible to increase the recovery by reducing the interval between injection and collection. In one experiment, for instance, in which collection was started 10 sec after the injection of 500 μg histamine, the recovery was 67 %. In another experiment the first sample of undiluted c.s.f. was collected 3 min after the injection of 500 μg of histamine in fourteen separate fractions of about 0·1 ml. each. The histamine concentration in the first three fractions was between 1:2300 and 1:2500 and then decreased quickly so that the concentration in the last four samples was about 1:20,000 only.

TABLE 1. Histamine content in successive samples of fluid collected from the cisterna magna in three cats. Collection started 2 hr after intraventricular injection of 500 μg of histamine

	Histamine content of samples (μg)		
Sample	Expt. 1	Expt. 2	Expt. 3
1	5·9 (1:102,000)	11·3 (1:44,000)	12·5 (1:16,000)
2	3·6	5·6	6·3
3	1·7	2·3	2·8
4	1·5	1·7	2·0
5	1·6	1·6	1·5
6	1·5	1·2	1·3
Total	15·8	23·7	26·4
Recovery (%)	3·2	4·7	5·3

When collection from the cisterna was begun 1 hr after injection of 500 μg of histamine, the recovery varied between 10 and 30 % (mean 20 %). Positional changes apparently did not affect the disappearance of histamine from the ventricles since the result was the same whether the head, fixed in the holder, was raised above the level of the body, the cat lying on its belly; or whether the head was kept at the same level as the body, the cat lying on its side. When collection was begun 2 hr after an injection of 500 μg histamine, the mean recovery was 4 % as shown in Table 1, which gives the amounts of histamine recovered in the six successive samples collected in three experiments. The concentration of histamine in the first samples of undiluted c.s.f. is given in brackets. When collection was begun 4 hr after an injection of 500 μg histamine, the recovery was 4·3 μg in the first, and 5·4 μg in the second experiment. The histamine content in the six consecutive samples of the second experiment

was 1·9, 1·5, 0·7, 0·5, 0·4 and 0·4 μg. The flow from the cannula in the cisterna was only small and the 1·9 μg was obtained in 0·2 ml. c.s.f.

Acetylcholine. When collection was begun 3 min after an intraventricular injection of 500 μg acetylcholine, the recovery was of the same order as in the experiments with histamine. It varied between 42 and 67 % (mean 56 %) and the concentration in the first sample of undiluted c.s.f. collected from the cisterna varied between 1:2000 and 1:8000 (mean 1:3600). In one experiment collection was begun 1 hr after the injection of 500 μg acetylcholine together with 100 μg eserine. The recovery was 3·6 %. This is less than the recovery obtained in corresponding experiments with histamine.

Recovery after death. The finding that neither histamine nor acetylcholine could be fully recovered from the cisterna when the collection was begun 3 min after their intraventricular injection, or even earlier, raised the question whether this incomplete recovery was the result of factors pertaining to the living cat or whether it would occur in the dead cat as well. In order to compare the recovery from the living and the dead cat the following procedure was adopted. An intraventricular injection of 500 μg of either histamine or acetylcholine was made in the living cat and the recovery determined after 3 min. One hour later the cat was killed by an overdose of pentobarbitone administered intraperitoneally. Ten minutes after death, the injection was repeated and the recovery again determined 3 min later. The results of two experiments with histamine and three with acetylcholine are given in Table 2. In all of them recovery was less in the dead than in the living cat. The difference was greater for histamine than for acetylcholine.

TABLE 2. Comparison in the living and the dead cat of the recoveries of histamine and acetylcholine from the cisterna magna 3 min after intraventricular injection of 500 μg

	Histamine or acetylcholine (μg) recovered from	
	The living cat	The dead cat
Histamine	384	183
Histamine	355	133
Mean	370	158
Acetylcholine	276	243
Acetylcholine	275	235
Acetylcholine	210	164
Mean	254	214

Recovery in dogs of the injected histamine and acetylcholine from the cisterna

The results are given in Table 3. In all but the last experiment two injections were made. Collection after the first injection was 3 min later; the second injection was given after an interval of about 1 hr.

Three minutes after the intraventricular injection of either 500 μg of histamine or 500 μg of acetylcholine the recovery was between 49 and 77%, whereas 1 hr after the injection the recovery varied between 3 and 12%. There was no significant difference between the recoveries of histamine and of acetylcholine.

TABLE 3. Recovery of either histamine or acetylcholine from the cisterna magna of dogs after intraventricular injections. In each experiment 500 μg of the same drug was injected twice with an interval of 1 hr. Collection started 3 min after the first, and 30 or 60 min after the second injection

Expt. no.	Drug injected	Eserine* 100 μg	Percentage recovery of the drug after 3 min	30 min	60 min
1	Histamine	.	55	.	5
2	Histamine	.	63	.	3
3	Histamine	.	62	19	.
4	Acetylcholine	+	49	.	7
5	Acetylcholine	+	77	.	12
6	Acetylcholine	+	.	.	4

* Injection of eserine (100 μg) preceded the injection of acetylcholine by 7 min in Expts. 5 and 6, while it was given together with acetylcholine in Expt. 4.

Recovery of the injected histamine from the subarachnoidal space

From the lumbar subarachnoidal space in dogs and cats. Fig. 6 illustrates a typical experiment on a dog. Collection was started 1 hr after the injection of 500 μg of histamine. The first sample of 0·6 ml. c.s.f. contained no histamine, nor did the second sample of 1·1 ml. fluid collected when the flow which had stopped was restarted by an intraventricular injection of 1 ml. of Tyrode solution. However, histamine appeared in the subsequent five samples collected whilst 9 ml. of Tyrode solution was injected intraventricularly, first in increasing and then in decreasing concentrations. The total amount assayed was 32·8 μg. It is clear that no histamine had reached the lumbar region before the lumbar flow was started and that part of the 32·8 μg must have come from the ventricles, passing down to the lumbar subarachnoidal space with the fluid injected intraventricularly. Therefore in another experiment the following procedure was adopted. After the flow had ceased, the needle was closed, a cannula inserted into the cisterna magna and several samples of cisternal fluid were collected, whilst Tyrode solution was injected into the lateral ventricle in order to wash out the histamine present in the ventricles. The cisternal cannula was then closed, the needle in the lumbar region opened and the flow from the lumbar region restarted by intraventricular injections of Tyrode solution. This alternate collection from the lumbar and the cisternal regions was then repeated. The result, illustrated in Table 4, shows that in spite of washing the ventricles through the cisternal cannula, some histamine reached the lumbar subarachnoidal space when, after the end

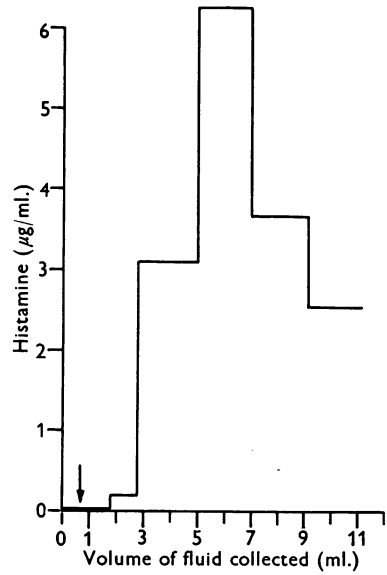

Fig. 6. Histamine content in successive samples of fluid obtained by lumbar puncture after an intraventricular injection of 500 μg of histamine into a 5·2 kg dog. Collection started 1 hr after the injection and continued for 35 min. From the arrow onwards Tyrode solution injected into the lateral ventricle during collection of each sample.

TABLE 4. Histamine content of the samples of fluid obtained by lumbar and cisternal punctures after intraventricular injection of 600 μg of histamine in the dog. Collection started 1 hr after the injection

Site of collection	Sample no.	Volume of Tyrode solution injected intra-ventricularly (ml.)	Volume of fluid collected (ml.)	Concentration of histamine in the collected fluid (μg/ml.)
Lumbar	1	Nil	0·6	Nil
	2	Nil	0·6	Nil
Cisternal	3	Nil	0·7	5·28
	4	2·0	1·0	2·40
	5	2·0	2·1	1·47
	6	2·0	2·0	1·50
	7	2·0	2·1	1·00
	8	1·0	1·7	0·76
	9	1·0	3·0	1·00
Lumbar	10	1·0	1·9	1·21
	11	1·0	0·5	1·80
Cisternal	12	1·0	1·3	0·22
	13	1·0	1·1	0·45
Lumbar	14	1·0	0·8	1·0
	15	1·0	0·4	0·75
	16	1·0	0·7	0·43
	17	1·0	0·8	0·41
	18	1·0	0·4	0·19
	19	1·0	1·2	0·13

of the ventricular washings, a flow was started from the ventricles to the lumbar region. The total amount of histamine in all lumbar samples collected was 5·2 μg. The finding that the concentration of histamine in the first lumbar samples collected after the ventricular washings (samples 10 and 14) was higher than that in the previous samples collected from the cisterna (samples 9 and 13) shows that part of the histamine at least was not coming from the ventricles but from the subarachnoidal space of the upper part of the spinal cord.

TABLE 5. Histamine content of the samples of fluid obtained by lumbar and cisternal punctures after intraventricular injection of 500 μg of histamine in the cat. Collection started 1 hr after the injection

Site of collection	Sample no.	Volume of Tyrode solution injected intra-ventricularly (ml.)	Volume of fluid collected (ml.)	Concentration of histamine in the collected fluid (μg/ml.)
Lumbar	1	Nil	0·2	1·1
Cisternal	2	Nil	0·9	60·0
	3	1·0	1·1	13·4
	4	1·0	1·1	6·1
	5	1·0	1·0	4·4
	6	1·0	1·0	2·9
	7	1·0	1·0	3·3
	8	1·0	1·0	2·85
Lumbar	9	1·0	0·2	8·3
	10	1·0	0·3	2·2
Cisternal	11	1·0	1·0	2·5
Lumbar	12	1·0	0·5	10·6
	13	1·0	0·45	11·8
Cisternal	14	1·0	1·1	0·45
	15	1·0	1·0	0·21
Lumbar	16	1·0	0·25	5·3
	17	2·0	0·15	1·73

In cats the first lumbar sample (0·2–0·3 ml.), collected 1 hr after an intraventricular injection of 500 μg, already contained 0·2–0·3 μg histamine; its concentration in subsequent samples collected during intraventricular injection of Tyrode solution rose and later fell again. Since part of this histamine must have come from the ventricles, the procedure of alternate collection from the lumbar and the cisterna regions was adopted. Such an experiment is illustrated in Table 5. The amounts of histamine collected from the lumbar region were higher than in dogs. This is not surprising, because the space of the ventricular system in cats is much smaller, yet the amount of histamine and the volume injected intraventricularly were the same in both species. The experiment shows that high concentrations of histamine must have reached the subarachnoidal space of the upper part of the spinal cord, because the concentration of histamine in the lumbar samples (samples 9, 12 and 16) was still high after it had fallen off in the cisternal samples (samples 8, 11 and 15).

From the epicerebral subarachnoidal space of the cat. When successive samples of about 0·1 ml. of c.s.f. were collected from the epicerebral subarachnoidal space starting 40–60 min after an intraventricular injection of 500 μg of histamine, the first sample again contained no histamine, or traces only. It appeared, however, in subsequent samples in increasing amounts. Two typical

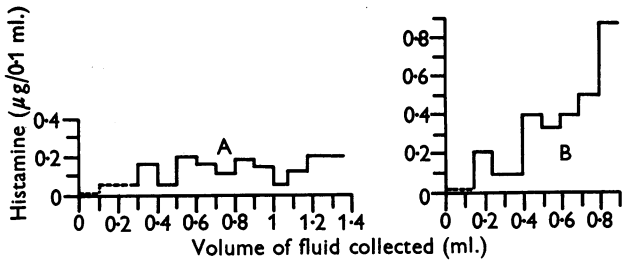

Fig. 7. Histamine concentration in successive samples of fluid obtained from the epicerebral subarachnoid space after intraventricular injection of 500 μg of histamine in two cats A and B. Collection started 1 hr after the injection. The dotted part indicates that the amounts of histamine were too small to be accurately assayed, probably they were smaller than indicated.

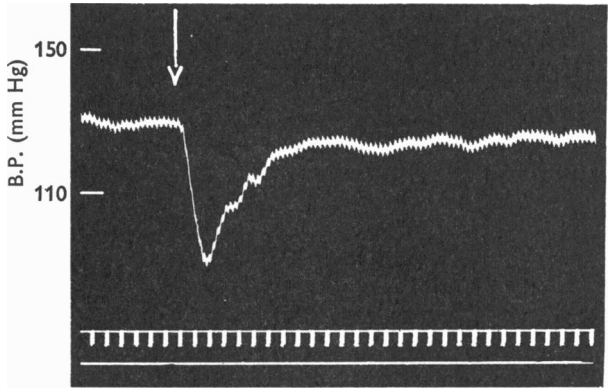

Fig. 8. Arterial blood pressure of a cat under chloralose anaesthesia. At the arrow (↓) intravenous infusion of histamine at a rate of 1·2 μg/min was begun and continued until the end of the tracing. Time marker, 30 sec.

experiments are illustrated in Fig. 7. The fluctuations in histamine concentration of successive samples were obtained in all experiments. The total amounts of histamine recovered in both experiments were less than 4 μg. In one experiment in which collection was started 20 min after the intraventricular injection, the first sample also was free from histamine but its concentration in subsequent samples rose to over 1 μg/0·1 ml.; in this experiment 1·7 ml. was collected in thirteen successive samples containing a total of 14·2 μg of histamine.

Absorption into the blood stream in cats of intraventricularly injected histamine

The transient fall in arterial blood pressure which often followed the intraventricular injection of 500 μg of histamine in cats was attributed to an initial absorption of a small portion of the injected histamine into the blood stream (see p. 171). The blood-pressure record, however, would not reveal whether or not absorption continued after the blood pressure had recovered, because a

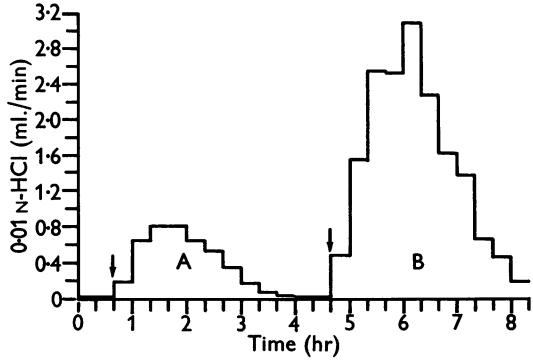

Fig. 9. Acid gastric secretion in a cat under chloralose anaesthesia with the intraventricular cannula implanted 2 weeks before the experiment. Comparison of the secretion in response to an intraventricular injection of 500 μg of histamine (at A) with that in response to a subcutaneous injection of 500 μg of histamine (at B); injections indicated by arrows.

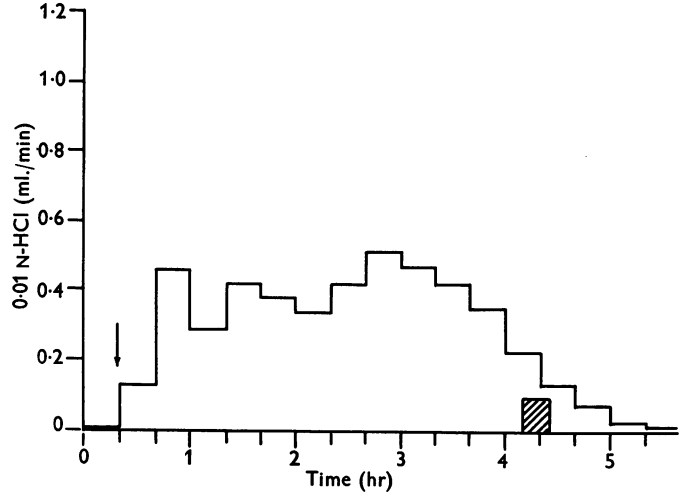

Fig. 10. Acid gastric secretion in response to an intraventricular injection of 500 μg of histamine in a vagotomized cat under chloralose anaesthesia. The intraventricular cannula had been implanted 1 week before the experiment. Time of injection indicated by the arrow (↓). Shaded area indicates washing out of histamine from the ventricles.

continuous intravenous infusion of histamine at a rate of about 1 μg/min also leads to a transient depressor effect only, as shown in the experiment of Fig. 8. The depressor effect resembles closely that seen in experiment Fig. 2C after an intraventricular injection of 500 μg of histamine. A slow continuous absorption of histamine into the blood stream, however, is easily detected by the acid gastric secretion.

Acid gastric secretion after intraventricular injection of histamine. Usually the first sample of fluid from the stomach, collected 20 min after an intraventricular injection of 500 μg histamine, already contained some hydrochloric acid. Secretion, however, reached its maximum after 40–80 min and then continued on a rather high level for periods varying between 1 and over 3 hr. When secretion began to decline it usually took 2–3 hr until it came to an end. To accelerate this decline, the ventricles were sometimes washed out. Typical experiments are illustrated in Figs. 9A and 10.

The secretion was not the result of a central action of the injected histamine either via the vagi or via the extravagal pathway postulated by Porter, Movius & French (1953). According to these authors stimulation of the posterior hypothalamus may lead to acid gastric secretion by release of ACTH, which would stimulate the suprarenals to release cortisone which causes acid gastric secretion as shown by French, Longmire, Porter & Movius (1953). Since the acid gastric secretion obtained by intraventricular injection of histamine occurred also after section of both vagi in the neck (see Fig. 10) and after removal of the suprarenals, the vagal and extravagal central secretory pathways have been excluded. The secretion must thus be a response of the oxyntic cells to the absorbed histamine.

Since the secretion was also obtained in experiments in which the cannula used for the intraventricular injection had been implanted 1–2 weeks before the actual experiment was performed, as in the experiments (Figs. 9, 10 and 13), the absorption was not from brain tissue freshly injured on insertion of the cannula.

In order to obtain more quantitative data about the rate of absorption, the acid gastric secretion produced by intraventricular injection of histamine was compared with that of histamine injected subcutaneously or slowly infused intravenously. For such a comparison the following facts have to be taken into account.

(1) When the same amount of histamine is given a second or third time, the acid gastric secretion increases with each administration. This happens whether the histamine is injected intraventricularly or subcutaneously, or infused intravenously. This is illustrated for two subcutaneous injections in Fig. 11.

(2) The secretion evoked by a slow intravenous infusion of histamine does not come to an end immediately when infusion stops, but continues for up to

1 hr at a relatively low level. Therefore, the end point of secretion after a subcutaneous or intraventricular injection of histamine does not coincide with the end point of absorption, and it is not possible in these circumstances to say for certain what part of the 'tail end' of secretion is still a sign of absorption.

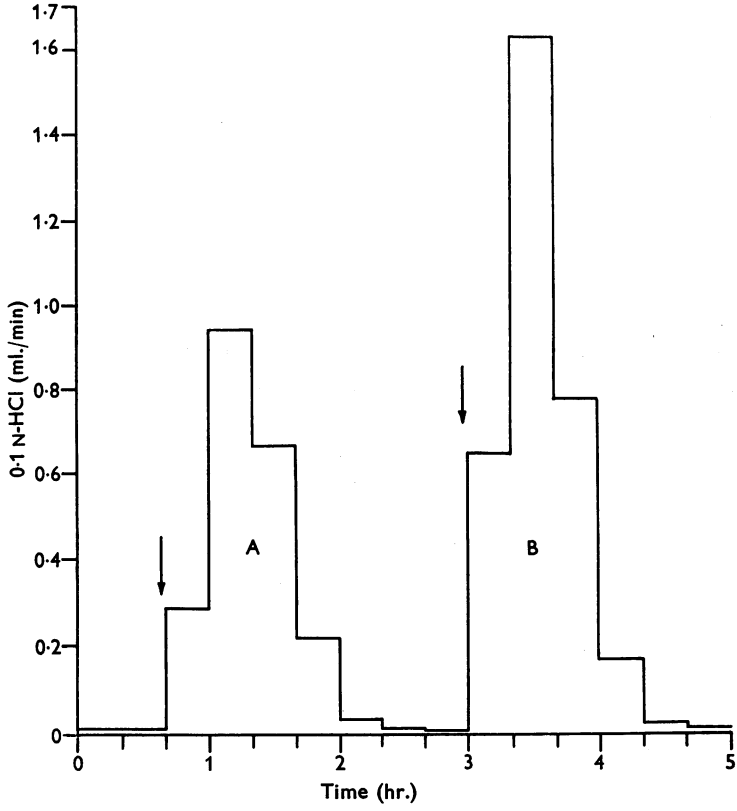

Fig. 11. Acid gastric secretion resulting from two successive subcutaneous injections of 250 μg histamine (at A and B) in a cat under chloralose anaesthesia. Injections indicated by arrows.

(3) If histamine is infused intravenously at a constant rate, the acid secreted increases in each successive 20 min sample. No definite peak of secretion was reached in these experiments even when infusion was continued for 4 hr. This means that when a peak of secretion is reached after an intraventricular injection of histamine, the rate of absorption may have been maximal some time before the peak was reached.

The maximal rate of secretion after an intraventricular injection of 500 μg of histamine was found to be less than that obtained after a subcutaneous injection of 500 (Fig. 9) and even of 250 μg of histamine, whether the sub-

cutaneous injection preceded or followed the intraventricular injection. This suggests that the rate of absorption after a subcutaneous injection of histamine is at least twice as great as after an intraventricular injection.

In several cats the maximal rate of acid gastric secretion in response to an intraventricular injection of 500 μg of histamine was compared with that obtained in response to slow intravenous infusions for 1 hr. The amounts of histamine infused per minute varied between 0·6 and 1·8 μg. Fig. 12 illustrates

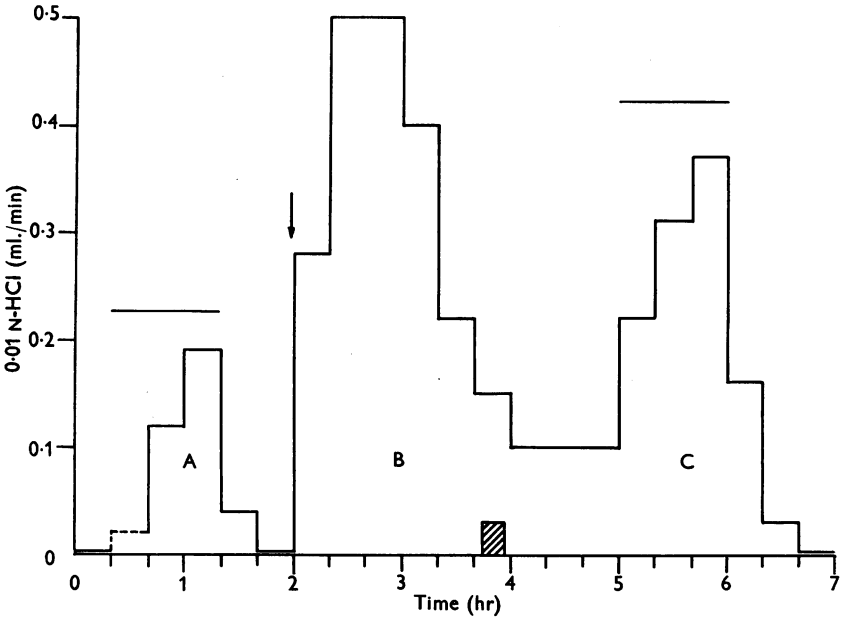

Fig. 12. Acid gastric secretion in a cat under chloralose anaesthesia. Comparison of the secretion in response to intravenous infusions of histamine 0·6 μg/min for 1 hr (at A and C) with that in response to intraventricular injection of 500 μg of histamine (at B). Time at which the intraventricular injection was given is indicated by an arrow and the duration of infusions by horizontal lines. Dotted part in A signifies that the acid secretion was probably less than indicated; shaded area indicates washing out of histamine from the ventricle.

an experiment in which the acid secretory response to an intraventricular injection of 500 μg of histamine was bracketed between the responses to two intravenous infusions of 0·6 μg histamine per minute. The secretion induced by the intraventricular injection reached a higher value irrespective of whether it followed or preceded the infusion. Therefore the maximal rate of absorption after intraventricular injection must be greater than 0·6 μg/min. Fig. 13 illustrates experiments on two cats in which the acid gastric secretion in response to an intravenous infusion of either 1·8 μg or 1·4 μg/min histamine reached higher levels than in response to a subsequent intraventricular injection of 500 μg of histamine. Thus the maximal rate of absorption after an

intraventricular injection of 500 μg histamine must have been less than 1·8 and 1·4 μg/min. In further experiments it was found that the maximal secretion produced by an infusion of 1·2 μg/min was equal or only slightly greater than that obtained after an intraventricular injection of 500 μg histamine. Thus the maximal rate of absorption of the intraventricular injection of 500 μg of histamine corresponded to an absorption of 1·2 μg histamine/min or slightly less.

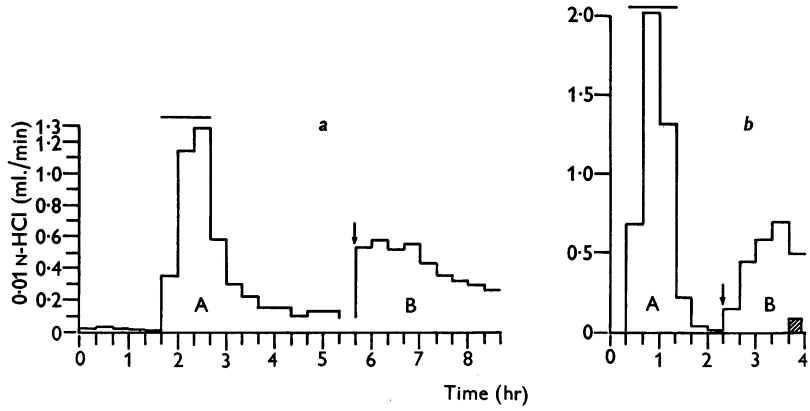

Fig. 13. Acid gastric secretion in two cats under chloralose anaesthesia. Cat of experiment (*a*) had the intraventricular cannula implanted 2 weeks before the experiment. Comparison of the secretion in response to 1 hr intravenous infusion (at A) of 1·8 μg histamine/min (*a*) and 1·4 μg histamine/min (*b*) with that in response to intraventricular injection of 500 μg of histamine (at B). Intraventricular injections indicated by arrows and duration of infusions by horizontal lines; shaded area indicates washing out of the histamine from the ventricle.

So far only the maximal rate of absorption has been considered. Estimates of the actual amounts of histamine absorbed can be obtained from the histograms of secretion by assuming that the maximal rate at which histamine enters the blood stream in the initial stages of increasing secretion is either 1·2 μg/min or 1 μg/min and that, from the moment there is a decline in the secretion, the rate of histamine entry into the blood stream diminishes gradually to 0. The period of maximal absorption is easily obtained from the histogram. The calculation for the absorption during the declining phase of secretion varies, however, according to whether the end point of absorption is taken to coincide with the end of secretion, or to have taken place one hour earlier, and according to whether one assumes a linear or an exponential decline of absorption. The values obtained for an exponential decline during the period of diminishing absorption are about 30% less than for a linear decline.

In Table 6 calculations have been made for five experiments. For each experiment two calculations are made. One is based on a maximal absorption of 1·2 μg/min and on the assumption that the end point of absorption coincides

with the end of secretion. This calculation errs on the high side. The other calculation is based on a maximal absorption of 1·0 μg/min and on the assumption that the end point of absorption occurs 1 hr before the end of secretion. This calculation errs on the low side. Both calculations are based on a linear decline of absorption; an exponential decline would reduce the values for the declining phase by about 30 % and these reduced values are given in brackets in the table as well.

TABLE 6. Absorption of histamine into the blood circulation after an intraventricular injection of 500 μg of histamine

	Initial period of maximal absorption			Period of declining absorption		
		Histamine absorbed				
Expt. of	Time (min)	(μg/min)	During initial period (μg)	Time (min)	Histamine absorbed (μg)	Histamine absorbed during both periods (μg)
Fig. 14	140	1·2	168	160	95 (66)	263 (234)
	140	1·0	140	100	50 (35)	190 (175)
						Mean 227 (205)
Fig. 10	40	1·2	48	260	157 (110)	205 (158)
	40	1·0	40	200	100 (70)	140 (110)
						Mean 173 (134)
	60	1·2	72	120	72 (50)	144 (122)
	60	1·0	60	60	30 (21)	90 (81)
						Mean 117 (102)
Fig. 13*a*	40	1·2	48	220	132 (92)	180 (140)
	40	1·0	40	160	80 (56)	120 (96)
						Mean 150 (118)
Fig. 12	40	1·2	48	140	84 (59)	132 (107)
	40	1·0	40	80	40 (28)	80 (68)
						Mean 106 (88)

In the first experiment of Table 6 the period of increasing gastric secretion lasted for 140 min. This was taken as the period of maximal absorption. Thus 168 or 140 μg would be absorbed at a rate of 1·2 or 1 μg/min respectively. The acid gastric secretion came to an end after a further 160 min so that for the end point of absorption either 160 or 100 min are taken. The method of calculation of the amounts absorbed during these 100 or 160 min periods is shown in Fig. 14. On the left, (A), the calculation is based on the assumption that at the beginning of the declining phase of absorption the rate was 1·2 μg/min and that the decline proceeded in a linear manner to 0 in 160 min. On the right, (B), the calculation is based on the assumption that at the beginning of the declining phase of absorption, the rate was 1 μg/min and that the decline proceeded in a linear manner to 0 in 100 min. The straight oblique lines show the decline in absorption values to zero in 160 or 100 min from the

initial values of either 1·2 or 1·0 μg/min respectively. By taking the points where the oblique line cuts the 10, 30, 50, 70, 90, 110, 130 and 150 min periods, the mean rate of absorption per minute for each successive 20 min sample is obtained. This rate is given beside the oblique lines and the figures represent μg/min. Further, by multiplying these values by 20, the μg of histamine entering the circulation during each successive 20 min sample are obtained (figures in brackets). By adding these amounts together, the total absorption expressed in μg histamine during the declining phase is obtained. This value

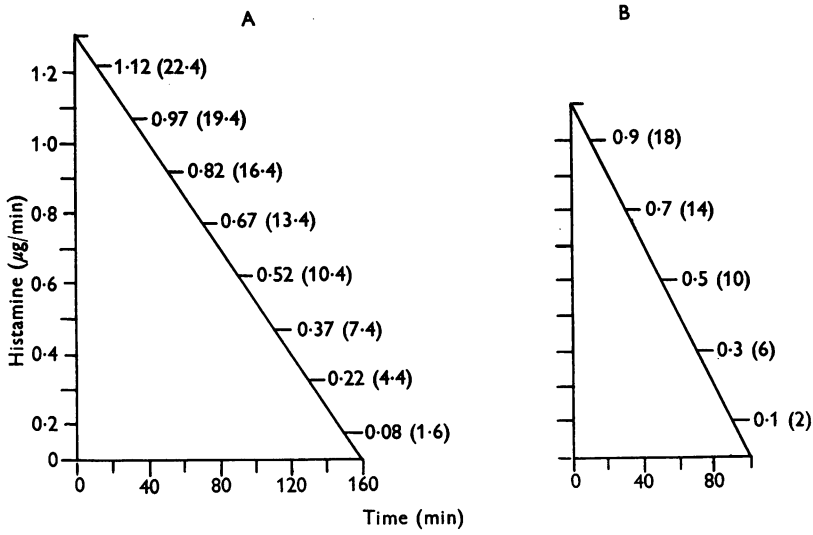

Fig. 14. Illustration of the calculation (for the first experiment Table 6) used for determining the amounts of histamine absorbed during the declining phase of acid gastric secretion, assuming linear decline. A: based on a maximal absorption of 1·2 μg/min and a declining phase lasting for 160 min. B: based on a maximal absorption of 1·0 μg/min and a declining phase lasting for 100 min. (For details, see text.)

is given in column 6 of Table 6. In the same way the values for the other experiments of Table 6 have been calculated. If we take the mean values of the two calculations for each of the five experiments of Table 6, between 21 and 45% of the injected histamine would have entered the circulation, or between 18 and 41% when the calculation during the declining phase is based on an exponential decline of absorption.

DISCUSSION

When 500 μg histamine or acetylcholine was injected into the lateral cerebral ventricle of the cat, and c.s.f. was collected from the cisterna magna—the collection being started 3 min or even earlier after the injection, and the ventricle washed during the collection—the injected histamine or acetylcholine

was not fully recovered. In fact, with this method, the recovery was only 40–77% or, on the average, about two-thirds of the injected histamine.

The problem arises as to why the recovery was not complete and where the remaining part of the histamine had gone. It is possible that the volume used for making the injection (0·25+0·25 ml.) pushed the histamine through the ventricular system, not only to the cisterna magna but also to parts beyond this region of the subarachnoidal space. When the collection from the cisterna magna was made 3 min after the injection in successive fractions of 3 drops each, the first fraction contained histamine in a concentration of about 1:2300. Even if the last drop of this fraction were already contaminated with the ventricular fluid, it is certain that, 3 min after the injection, the c.s.f. in the cisterna magna contained a high concentration of histamine. Since the cisterna magna communicates freely with the regions of the subarachnoidal space surrounding the base of the brain and upper part of the cervical cord, it may be assumed that some of the unrecovered histamine had diffused into these regions and escaped recovery by cisternal puncture and subsequent washing of the ventricle. The experiments on acid gastric secretion show that histamine reaches the general blood circulation within the first 20 min after intraventricular injection and must thus have traversed a greater distance of the subarachnoidal space than that adjoining the cisterna.

The finding that the recovery was even less in the dead than in the living cat could be explained by a greater escape of the injected drug into parts of the subarachnoidal space adjoining the cisterna magna. The volume of the cranial box does not change, nor does that of the brain. The brain, however, does not occupy the whole of the cranial box. The remaining space is taken up by the c.s.f. and the blood vessels. During dilatation of the vessels the subarachnoidal space taken up by the c.s.f. decreases, and during contraction it increases. Thus in order to understand why after death more of the injected histamine escapes into the regions of the subarachnoidal space not drained by the method used for collection from the cisterna magna, it is only necessary to assume that after death there is a reduction in the volume taken up by the vessels and consequently a relative increase in the space occupied by the c.s.f.

The experiments on acid gastric secretion after intraventricular injection of histamine show that part of the injected histamine is absorbed into the blood stream. When the absorption of histamine into the blood stream is correlated with its disappearance from the cerebral ventricle, it is found that, during the first hour after an intraventricular injection the rate of absorption is greatest and so is the rate of disappearance. The time course of maximal absorption also appears to parallel the maximal rate of disappearance of histamine from the ventricles, particularly if we take into account that the injected histamine has to traverse a relatively large area of the subarachnoidal space before it is absorbed into the endocranial venous sinuses. However, the parallelism breaks

down when the actual amounts disappearing from the ventricles and those being absorbed into the blood stream are correlated, because about 80% disappears during the first hour and about 99% within 4 hr, whereas the absorption as calculated from the total period of acid gastric secretion accounts for less than half of the injected histamine only. About the fate of that part of histamine which is not accounted for by absorption into the blood stream it is at present possible only to speculate. A certain amount is certainly accounted for by the histamine reaching the subarachnoidal space around the brain stem and upper spinal cord from where it would be absorbed only very slowly, but part of the unrecovered histamine may have been taken up by the brain substance or may have been destroyed enzymatically on its way to the venous sinuses. This pertinent problem has not been examined.

One of the reasons for performing the experiments presented in the present paper was to find out whether the long-lasting effects observed in unanaesthetized animals after intraventricular injection of the drugs were associated with the persistence of the drugs in the ventricles. Feldberg & Sherwood (1954) found that within an hour after an intraventricular injection of 150–200 μg of histamine, in cats, the main effects became weaker and the recovery, though not complete, started within this period. In the present experiments it was found that 1 hr after the intraventricular injection of 500 μg of histamine about 20% of the histamine could still be recovered. There is thus, as far as histamine is concerned, no reason to assume that the action it produces persists after the histamine has left the ventricular spaces.

SUMMARY

1. In anaesthetized cats and dogs histamine or acetylcholine was injected into the cerebral lateral ventricle and, at various times after the injection, the cerebrospinal fluid was removed from the cisterna magna, the ventricular spaces were washed out by repeated ventricular injections, the washings were also collected and the samples assayed for histamine or acetylcholine respectively.

2. In cats the mean recoveries of histamine from the cisterna were 63% when collection was started 3 min, 20% when started 1 hr, and 4 and 1% when started 2 and 4 hr respectively after the injection. In cats acetylcholine disappeared earlier from the ventricles since only a small percentage could be recovered from the cisterna 1 hr after the injection.

3. In dogs the recoveries of histamine or acetylcholine from the cisterna were between 49 and 77% when collection was started 3 min, and between 3 and 12% when collection was started 1 hr after the injection.

4. One hour after an intraventricular injection of 500 μg of histamine none, or only small amounts, were detected in the c.s.f. from the lumbar or epicerebral subarachnoidal spaces.

5. The intraventricular injection of 500 μg of histamine into the anaesthetized cat caused acid gastric secretion, which could be attributed to absorption of the histamine into the blood stream.

6. By comparing the acid gastric secretion with that of a slow intravenous infusion of histamine the maximal rate of absorption after an intraventricular injection of 500 μg of histamine was found to be between 1 and 1·2 μg/min.

7. From the histograms of acid gastric secretion the amounts of histamine absorbed into the blood stream were calculated to be less than half the amount injected intraventricularly. Part of the unaccounted histamine might be the histamine lingering in the subarachnoidal spaces around the brain stem and upper spinal cord from where it would be absorbed slowly.

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