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THE EFFECT OF GENERAL ANAESTHESIA ON THE SEROTONIN LEVELS IN BRAIN, BLOOD, KIDNEY AND SMALL INTESTINE OF THE EXPERIMENTAL ANIMALS

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INTRODUCTION:

Serotonin (5-hydroxytryptamine) is an active amine which is actually locally synthesiced in tissues rather than transmitted from one organ to the other. It is a potent vasoconstrictor agent and smooth muscle stimulant. It causes an increase in peripheral vascular resistance as well as coronary vasospasm (Goodman and Gillman, 1976).

5-hydroxytryptamine (5-HT) has a complex action on blood pressure. Its action starts by a brief depressor phase, probably due to coronary chemoreflex: followed by a pressor phase due to its direct action. Finally it has a depressor phase due to direct dilator action.

The action of serotonin on the central nervous system was studied on animals, and it was found to cause some dizziness with a variety of stimulant and depressant actions causes of which are uncertain (Goodman and Gillman, 1976). It has a stimulant action on peripheral nerves. Sherwood (1954) found that 5-HT, produced muscle weakness when injected intraventricularly. Animals in his experiment attained a sleeping posture with some degree of catatonia. Cutis & David (1962) found that 5-HT blocked transmission through the lateral geniculate nucleus.

Lida Swafford (1966) found that serotonin had a direct relation to stress conditions. Serotonin level was elevated in the blood in pulmonary embolism. Also serotonin experimentally produced pulmonary artery hypertension. Anderson & Bonnycastle (1960) and Bonnycasle et al (1962) observed an increase of the level of brain serotonin during anesthesia with ether and other central nervous system depressants. Neff et al (1967) and Diaz et al (1968) found an increase of cerebral serotonin level in animals during anaesthesia using different anaesthetics. Lida Swafford (1966) postulated a direct relationship between general anaesthesia and the level of serotonin in blood and she suggested that the use of antiserotonin agents prophylactically during general anaesthesia might result in reduction of the incidence of such complications.

In the present work we planned to subject experimental animals (rabbits) to four different types of inhalation anaesthesia using, ether, halothane, trilene and chloroform and estimate the serotonin level in blood, brain, kidneys and small intestine during both the excitatory as well as the surgical stages of anesthesia. We aimed to establish a relationship between the serotonin level and the state of anaesthesia and to observe variations between the different types of general inhalation anaesthetics.

MATERIAL AND METHODS:

Animals used:

1- Male albino rabbits weighing between 1.5 and 2 Kg. were subjected to general anaesthesia by inhalation after a fasting period of 12 hours. Eight groups of animals were used; two groups for each of

TABLE I. EFFECT OF DIETHYLETHER, HALOTHANE AND TRILENE ANAESTHESIA ON THE LEVELS OF SEROTONIN IN BRAIN, BLOOD, KIDNEY & SMALL INTESTINE AT THE EXCITATORY/ STAGE AND AT AFTER 30 MIN OF SURGICAL STAGE

	EXPERIMENT	BRAIN	BLOOD	KIDNEY	INTESTINE
A.	NORMAL	276 ± 8	4442 ± 74	48 ± 5	3093 ± 145
B.	DIETHYLETHER TREATED				
	(a) Excitatory stage	321 ± 14	4388 ± 87	128 ± 8	3243 ± 109
	(b) After 30 min of surg. stage	460 ± 22	5537 ± 107	155 ± 9	4500 ± 203
C.	HALOTHANE TREATED				
	(a) Excitatory stage	395 ± 13	4497 ± 74	43 ± 1	3231 ± 57
	(b) After 30 min of surg. stage	446 ± 25	4286 ± 95	93 ± 3	3583 ± 148
D.	TRILENE TREATED				
	(a) Excitatory stage	506 ± 16	5189 ± 262	111 ± 8	3623 ± 193
	(b) After 30 min of surg. stage	550 ± 23	6341 ± 149	231 ± 7	4417 ± 218
E.	CHLOROFORM TREATED				
	(a) Excitatory stage	308 ± 13	4857 ± 189	86 ± 6	2989 ± 76
	(b) After 30 min of surg. stage	317 ± 10	4760 ± 279	114 ± 3	3306 ± 105

THE VALUES ARE MEAN ± S E DERIVED FROM 7 - 8 DIFFERENT EXPERIMENTS AND ARE EXPRESSED AS ng/g tissue.

Table (2) Effect of diethyl ether anaesthesia at the excitatory stage on serotonin level in rabbits tissues, brain, blood, kidneys and small intestine in nan/gm.

No.	Brain	Blood	Kidneys	Intestine
1	375	4720	I70	3600
2	350	4IC0	I00	3I00
3	300	4200	I25	3000
4	275	4400	I25	3I00
5	300	[illegible]	I25	2900
6	300	4200	I35	3600
7	350	4600	II5	3400
Mean	32I.4	4388.57	I27.86	3242.86
S. E.	±I3.83	±87.48	±8.I5	± I08.79
S. D.	±36.59	±23I.47	±2I.57	± 287.85
T.	±2.77	±0.469	±0.36	±0.69I
P.	<0.05	>0.05	<0.05	>0.05

Results compared with contron group (table).

Table(3) The effect of diethyI ethyI ether anaesthesia after 30 min of surgical stage on the serotonin level in rabbits tissues, brain, blood kidneys and smaII intestine in nan/gm.

No.	Brain	Blood	Kidney	Intest
I	380	5400	90	4200
2	500	5640	I35	4080
3	490	5400	I35	4360
4	500	5600	I25	4000
5	400	6I00	90	5680
6	440	5360	9054	4300
7	460	5200	I40	4480
Mean	460	5537.14	155	4500
S. E.	±20.36	±I06.5a	±8.99	±202.86
S. D.	±59.I6	±20f.99	±23.8	±5I6.78
T.	±7.698	±a.425	±6.489	±5.627
P.	< 0.05	< 0.05	< 0.05	< 0.05

Mean values compared with control group (tableI).

Table (4) Effect of halothane anaesthesia at the excitatory stage on the serotonin IeveI in rabbit's tissues, brain, blood, smaII intestine & kidneys in nan/gm.

No.	Brain	Blood	Kidney	Intestine
1	325	4720	50	3200
2	390	4320	40	3120
3	390	4560	40	3I20
4	420	4760	45	3300
5	430	4480	40	3440
6	410	4240	40	3400
7	400	4400	45	3040
Mean	395	4497.14	42.86	3231.43
S. E.	±I2.95	±73.98	±1.49	±57.54
S. D.	±34.28	±195.76	±3.93	±152.25
T.	±7.725	±0.521	±1.173	±0.877
P.	< 0.05	>0.05	>0.05	>0.05

Table (5) The effect of halothane anaesthesia after 30 min of the surgicaI stage, on serotonin IeveI in rabbit's tissues,brain, bIood, kidneys and smaII intestine, in nan/gm.

No.	brain	blood	Kidney	Intestine
I	550	4I60	90	4I20
2	5I0	4440	95	4000
3	450	4240	II0	3400
4	380	4360	90	3440
5	450	4480	85	3I60
6	380	4520	90	3800
7	400	3800	90	3I60
Mean	445.7I	4286.7I	92.86	3582.86
S. E.	±24.77	±94.58	±3.08	±I48.08
S. D.	±65.54	±25.26	±8.09	±391.82
T.	±6.47	±I.30	±7.75	±2.35
P.	< 0.05	>0.05	<0.05	≤0.05

the general anaesthetic used, diethyl ether, halothane, trilene and chloroform; for each drug, a group of animals was examined during the excitatory stage and the other during the surgical stage of anaesthesia. A ninth group was examined as control.

2-Albino rats of either sex weighing 100 and 150 gm. were used for bioassay of the level of serotonin in various tissues. Animals were kept separate and fasted for 24 hours, then they were sacrificed and a longitudinal strip of the gastric fundus was removed and placed in an organ bath for bioassay.

INDUCTION OF GENERAL INHALATION ANAESTHESIA:

Ether was applied in a concentration of 4% and halothane was used in a concentration of 0.75%. Each of these drugs was used by EMO apparatus. On the other hand trilene and chloroform were applied using a mask by open method. Rabbits were observed till they reached the required stage of anaesthesia. Animal was then sacrificed and samples of brain, kidney, small intestine and blood were taken separately for assay.

EXTRACTION OF SEROTONIN FROM TISSUES:

Each tissue was separated, washed, homogenized (using a measured quantity of acetone 95%), and then macerated for one hour at room temperature. The substance was then filtered twice through Whatman No. 1 filter. The residue was then dried and suspended in a measured amount of saline for bioassay.

One ml. of blood was mixed with one ml. of distilled water and 38 ml. of acetone and shaken well to be haemolysed. The haemolysed material was macerated, filtered and extracted as before (Amine et al. 1954).

BIOLOGICAL ASSAY OF SEROTONIN IN RABBITS TISSUES:

The method described by Vane (1957) and improved by Uuspaa (1962) using the rat fundal strip preparation was used. A longitudinal muscle strip was separated from the rat gastric fundus and stretched in the organ bath (Lin & Yeoh, 1965). The maximal stretch of the longitudinal muscle strip was obtained for best sensitivity (Offermier & Ariens, 1966). This was bathed in magnesium free Kreb's solution with ample flow of air for oxygenation.

Each tissue extract was assayed against standard serotonin solution. Results were recorded on a moving drum.

RESULTS

In the present work we estimated the serotonin levels in the brain, blood, kidney and small intestine of animals induced with ether, halothane, trilene or chloroform; both during the excitatory state and during the stage of surgical anaesthesia.

Using diethyl ether as anaesthesia, the mean serotonin level in the brain showed a rise from 276.875 ng/g. in the normal control group to levels of 321.4 and 460 ng/g. during the excitatory and surgical stages respectively (Table 1 and Fig. 1) Halothane, on the other had resulted in lesser increase to level of 395 and 445.71 ng/g. during above mentioned stages respectively. The most dramatic rise was encountered during the use of trilene as a general anaesthetic when the brain levels of serotonin rose to 505.71 and 550 ng/g. in the two stages of anaesthesia respectively. The chloroform caused a very slight increase of serotonin level in the brain as the mean levels in both stages of anaesthesia were 307.86 and 317.14 ng/g.

By studying the changes in serotonin level in the blood in both stages of anaesthesia using each of the four mentioned anaesthetic agents we found that the most dramatic reise of blood serotonin occurred during the use of trilene as the level rose to 5188.57 and 6348.57 g/g. as compared to the normal level of 4442.5 g/g. Ether anaesthesia also resulted in a rise of the level of blood serotonin to 5537.14 in the surgical stage; while halothane did not show a significant variation from normal and chloroform resulted in a much less rise 4857.14 g/g. in the excitatory stage.

DISCUSSION

The effect of inhalation anaesthesia using ether, halothane, trilene and chloroform, on the serotonin level in blood, brain, kidneys and small intestine was experimented in the present work using albino rabbits for anaesthesia and estimating the serotonin level by bioassay.

Serotonin level in the brain rose to a significant level during anaesthesia, both at the excitatory stage and after 30 min. in the surgical stage by all four types of inhalation anaesthesia (Fig. 1A). The peak was noted during the use of trilene and ether being second to it. Rise being 198.7% with trilene, 166% with ether while it was 160% with halothane and 114% with chloroform.

These results coincide with those of Diaz et al (1968) who observed a rise of more than 100% with anaesthesia in brain serotonin level. Diaz followed his experiment for four hours and he noticed that the blood serotonin level during halothane anaesthesia showed a gradual decrease while with ether it was almost steady. He and other workers (Bonnycastle et al 1962) suggested the rise due to increased synthesis rather than impaired oxidation.

The blood level of serotonin also rose during inhalation anaesthesia. The present increase being 124.6%, 101%, 142% and 107% during ether, halothane, trilene and chloroform anaesthesia respectively. The highest level noted was with trilene while halothane showed an insignificant change (Fig. 1B).

Table (6) Effect of trilene anaesthesia at the excitatory stage on serotonin level in rabbits tissue brain, blood, kidney and small intestine, in nan/gm.

No.	Brain	Blood	Kidney	Intestine
1	490	5560	150	3120
2	570	5960	110	4640
3	460	5300	105	3640
4	400	4160	80	3120
5	550	5980	130	3620
6	430	4600	105	3720
7	520	4760	115	4500
Mean	505.71	5180.57	117.57	3622.86
S. E.	±16.16	±262.08	±6.29	±193.02
S. D.	±42.76	±693.47	±21.93	±510.74
T.	±12.05	±2.73	±6.68	±2.18
P.	<0.05	<0.05	<0.05	<0.05

Table (7) The effect of trilene anaesthesia 30 min of surgical stage on serotonin level in rabbits tissue brain, kidney and small intestine, nan/gm.

No.	Brain	Blood	Kidney	Intestine
1	580	6160	260	4920
2	520	6080	210	5200
3	660	6800	250	4120
4	530	6080	215	4480
5	460	6560	220	3480
6	560	5880	230	4080
7	540	6080	230	4640
Mean	550	634'.57	230.71	4417.14
S. E.	±23.19	±148.81	±6.94	±218.17
S. D.	±61.37	±393.76	±18.35	±577.28
T.	±11.11	±11.45	±21.58	±5.04
P.	<0.05	<0.05	<0.05	<0.05

Table (8) Effect of chloroform anaesthesia at the excitatory stage on the serotonin level in rabbits tissue, brain, blood, kidney and small intestine in nan/gm.

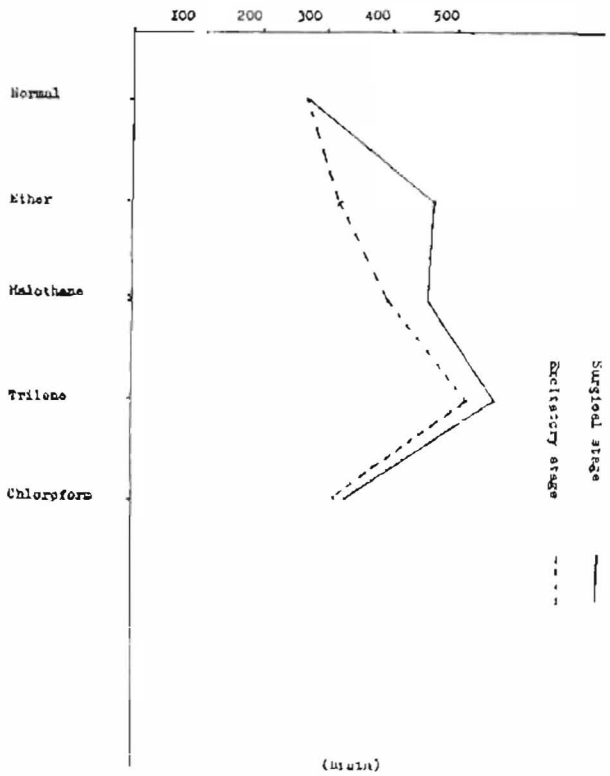
No.	Brain	Blood	Kidney	Intestine
1	290	4360	110	3080
2	380	5560	70	3160
3	290	5200	90	300
4	200	4600	65	2960
5	205	4960	80	2560
6	310	5000	105	3120
7	320	4180	80	3040
Mean	307.86	4857.14	85.71	2988.57
S. E.	±13.18	±154.14	±6.4	±75.84
S. D.	±43.86	±407.23	±16.94	±200.95
T.	±2.601	±2.088	±4.621	±0.639
P.	<0.05	<0.05	<0.05	>0.05

Table (9) Effect ofchloroform anaesthesia after 30 min in surgical stage on the serotonin level in rabbits tissues, brain, blood, kidneys and small intestine,in nan/gm.

No.	Brain	Blood	Kidney	Intestine
1	350	5800	115	3620
2	330	4080	110	3000
3	300	5560	110	3480
4	325	4060	105	3020
5	305	4000	120	3200
6	270	4000	115	3660
7	340	5800	115	3160
Mean	317.14	4760	114.28	3305.71
S. E.	±10.34	±279.46	±2.54	±104.89
S. D.	±27.36	±739.46	±6.726	±277.54
T.	±3.061	±1.098	±12.074	±1.179
P.	<0.05	>0.05	<0.05	>0.05

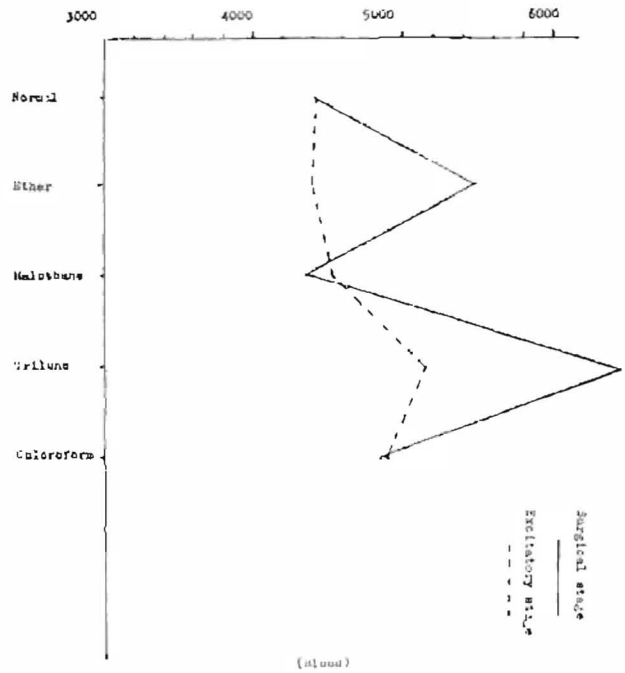
Serotonin level in the tissues by bioassay, mcg/gm.

(Fig. A)



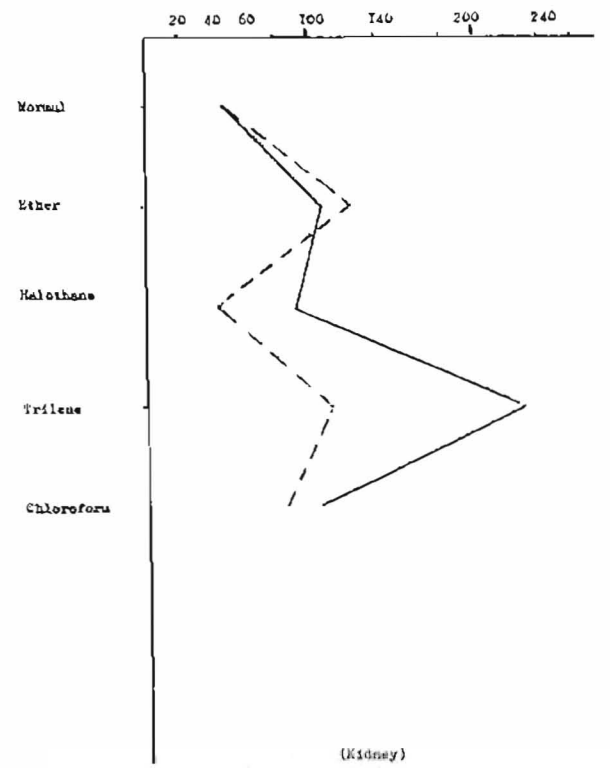
Serotonin level in the blood of rabbits mcg/ml.

(Fig. B)



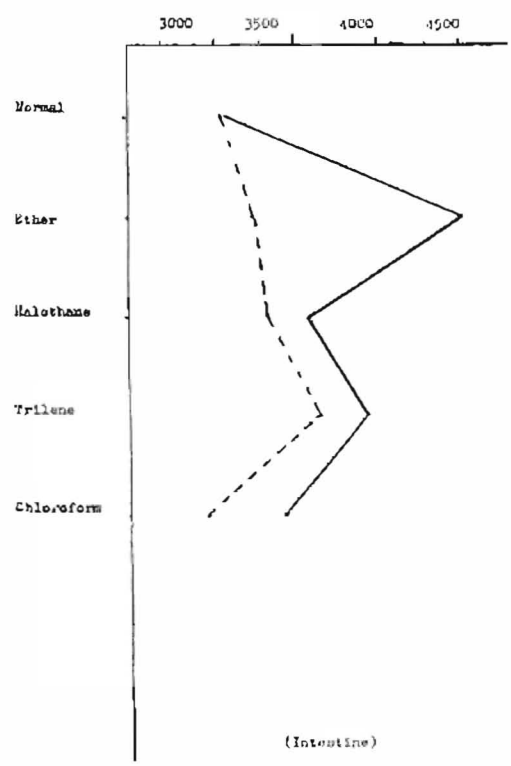
Serotonin level in the kidneys of rabbits mcg/gm.

(Fig. C)



Serotonin level in the intestine of rabbits mcg/gm.

(Fig. D)



By estimating the kidney and small intestine level of serotonin during anaesthesia the highest levels were noted during the use of trilene inhalation anaesthesia then diethyl ether (Fig. C & D).

Tricholoroethylene (trilene) is disreputed for its complications. Convulsions were reported during its use as an inhalation anaesthesia specially with children (Goodman & Gillman, 1976). Dysrhythmias, sinus tachycardia and sometimes heart failure (Edwards et al 1954) are also reported to have occurred. This may be correlated with the significantly high level of serotonin in blood and brain during its use as a general anesthetic as observed during the present work and by other investigators (Diaz et al, 1968 & Bonnycastle et al, 1962).

Diethyl ether has no significant toxic action on the heart, but the occurrence of post operative pulmonary embolism in some cases may be related to the elevation of serotonin level in the blood during anaesthesia using ether. This was observed by Lida Swafford (1966) who suggested the use of prophylactic antiserotonin agents in such cases to lower the incidence of such complications. This may be a point to be considered in further studies.

Chloroform is known to have many toxic effects on the heart muscle causing irritability, fibrillation and possibly heart failure, (Hill, 1932). It is not commonly used as a general anaesthetic.

Halothane as a general inhalation anaesthetic has the least number of side reactions. It has a direct action on the vascular smooth muscles with slight reduction of the peripheral resistance and blood pressure (Deutsch et al, 1962). This is to some extent counteracted by the action of serotonin in the blood. Halothane on the other hand increases the cerebral blood flow (Wooldman et al, 1965). It also results in skeletal muscle vasodilation (Black & McArdle, 1962). Halothane anaesthesia during the present study resulted in a slight rise of serotonin levels in brain and blood (Fig. 1, A & B).

As regards the rise in serotonin levels in kidneys and small intestine observed during inhalation anaesthesia (Fig. C & D) this may be partly related to the vascular action of serotonin and partly due to the stress condition.

SUMMARY

The serotonin level in blood, brain, kidneys and small intestine of rabbits was examined during the excitatory and surgical stages of inhalation anaesthesia using ether, halothane, trilene and chloroform. The rise was most marked with trilene next was ether and correlated with the complications expected in each case.

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وما أوتيتم من العلم إلا قليلا