CHLOROFORM: ITS EFFECTS IMMEDIATE AND REMOTE.

An experimental study.

A thesis presented for the degree of

Doctor of Medicine

by

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May, 1914.

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C O N T E N T S

Appendix IV. Details of an experiment on a dog showing the influence of depth of chloroform anaesthesia upon the effects of Carbon Dioxide. page 112.

P R E F A C E

The very extensive use that is made of chloroform as an anaesthetic, in Scotland at least, is, no doubt, in large measure due to the relative ease of its administration.

Notwithstanding the observance of due caution, deaths occasionally happen, sometimes before or during operations, and sometimes days, if not weeks afterwards. The cause of these fatalities has been the subject of experimental investigation for some years, and in the Thesis presented herewith, the author gives the results of his own and other work upon this subject.

The original work was commenced in 1907 so that part at least of the facts detailed are now common knowledge. A copy of each of the Author's publications on the subject is presented.

These are as follows:-

1. On the Histological Changes in the Liver and Kidney after Chloroform administered by different channels.

   Proc.Roy.Soc.Edin., Vol.XXIX. 1908-9, p.420.

2. The Influence of Chloroform when repeatedly administered in small doses.

   The Lancet, Jan.21st. 1911.

3. The Effects of Chloroform.

   Glasgow Medical Journal, July, 1912.

4. The Distribution of Chloroform in the blood.

   The Lancet, July 27th. 1912.

   (with Miss D.E.Lindsay)

5. The Influence of Carbon Dioxide on the heart in different degrees of anaesthesia.

   Journal of Physiology Vol.xlvii, 1913, p.393.

   (with Dr.E.P.Cathcart)

6. Chloroform Anaesthesia in the light of Physiological Research.

   Glasgow Medical Journal, January, 1914.

7. On the dangers of light Anaesthesia.

   Glasgow Medical Journal, April 1914.

   (with Dr.E.P.Cathcart).

METHODS OF ADMINISTRATION.

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The method most commonly adopted in the administration of chloroform is that of inhalation of the drug. A porous mask is placed over the mouth and nostrils of the subject, and the drug is dropped on from time to time, until anaesthesia is produced. In the more scientific methods an apparatus, such as that of Harcourt (1. *) is used by which means accurately measured percentages of chloroform vapour may be given to the subject. This method is of great importance, as the rapid administration of high percentages of chloroform vapour by inhalation is followed frequently by symptoms of acute chloroform poisoning. This is considered on page (63). Unfortunately such methods are not employed as generally as the interests of patients would warrant.

In the method most generally adopted there is no means of estimating the amount of chloroform that is being inhaled, although it is generally taken that if the mask is kept ½ inch distant from the face the percentage inhaled does not exceed 2.

In

* (the numbers in parenthesis refer to the bibliography at the end)

In practice this works out fairly well, although the alternation of periods of very deep anaesthesia with periods of very light anaesthesia cannot be considered to be devoid of danger. See also page (67-8). This necessarily occurs when the anaesthetic is given by the method referred to, and chloroform is given until the conjunctival or the pupillary reflex to light disappears, under the anaesthetic and the administration ceases until this returns - often until movement of the subject indicates that the anaesthesia is no longer "full".

It is impossible to over-estimate the importance of administering chloroform "smoothly", and later (page) I refer to much work proving conclusively that the dangers of chloroform (and other) anaesthesias are increased by the irregular administration of the drug.

Recently an attempt has been made to introduce an intravenous method of giving chloroform by Giani and others (2) similar to that used in ether admministration. This method approximates closely to that adopted by me in administering the drug subcutaneously (page 68) and consequently my findings after such injections may be applied to the intra-venous injections, due regard being paid to the fact that the intra-venous injections are likely to have a

a more rapid and far-reaching result as the injected substance passes at once and not gradually into the blood.

Lastly chloroform is given "per os". When administered in this way chloroform is not given to produce anaesthesia, but generally for its sedative qualities in the form of chlorodyne (containing 6 per cent of chloroform) or for its sedative and flavouring qualities as aqua chloroformi in mixtures. As chlorodyne it is taken in relatively large quantities in various cough mixtures and lozenges. Crichton Browne(3.) has referred to this and the results have been investigated in animals. The data appear on page (4[illegible]).

In considering the action of chloroform in its relation to medicine, observations must be made after it has been (a) inhaled, (b) injected and (c) given "per os". In the work presented now, an attempt is made to show how each method of administration effects histological structure of the organs and therefore metabolism and health.

ASSUMPTION OF CHLOROFORM.

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It has been conclusively shown by Pohl (4) Nicloux (5) Moore and Roaf (6) and Buckmaster and Gardner (7) that when Chloroform is associated with the blood the bulk of the drug is held by the red corpuscles. According to Nicloux's most careful estimations 88 per cent of the chloroform in the blood is held by the corpuscles, and only 12 per cent by the plasma. It appears, then, highly probable that in chloroform anaesthesia the transport from and to the surface of the lungs is a function of the red corpuscles. The particular element in the red corpuscles which attaches the chloroform to itself is not so clearly established. The compound or association of the chloroform with the red corpuscle must be loose if the blood is to pass the chloroform on to the tissues. Moore and Roaf (6) hold that the porteins form unstable compounds with the chloroform. They argue that "since proteins build up the living protoplasm, chloroform and other anaesthetics must form similar unstable compounds with protoplasm, and that anaesthesia is due to the formation of such compounds which limit

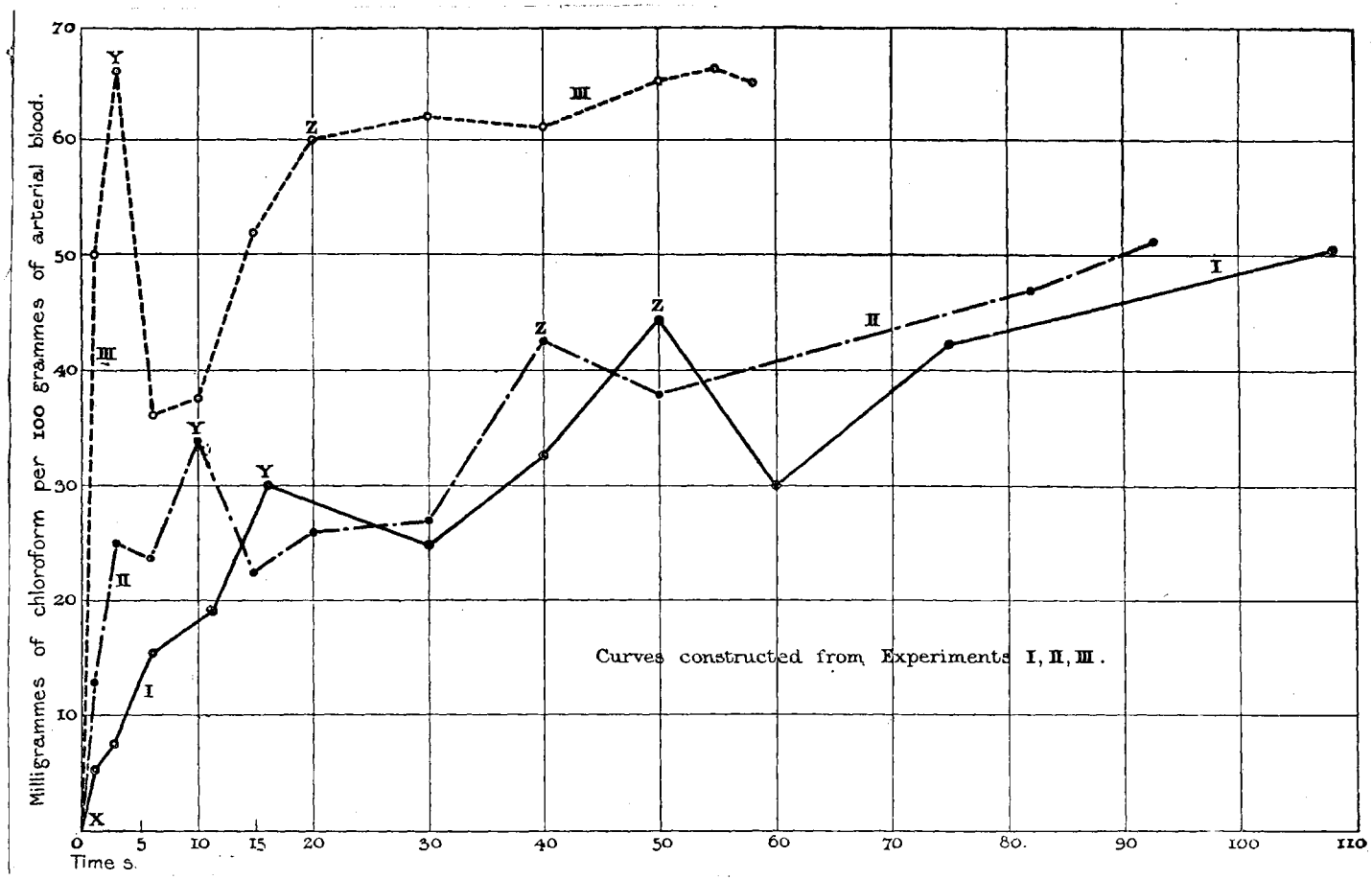
limit the chemical activities of the protoplasm. On account of the instability of the compounds, these remain formed only so long as the pressure of the anaesthetic in the solution is maintained". Others (Nicloux and Meyer) hold that the "lipoid" element in the corpuscles, plasma, and tissues is responsible for the fixing of the chloroform, so long as the vapour pressure is sufficient. The term "lipoid" is held to mean those fat-like constituents of animal or vegetable cells which can be extracted by means of ether or similar solvents (Rosenheim 8). Meyer (9) has postulated the hypothesis that all bodies capable of dissolving fats possess, in a greater or less degree, anaesthetic properties. This suggestion as to the importance of the "lipoids" in anaesthesia has been supported by the experiments of Nicloux (10) who showed that, if an animal is anaesthetised with chloroform and killed when fully anaesthetised, the nervous tissue of the brain and spinal cord contained a larger proportion of chloroform than any other tissues - the blood excepted. In further examining this point, Nicloux and Mlle.Frison (11) found that the white matter held more of the anaesthetic than the grey, and that the different power of fixation of chloroform varied with the proportion

proportion of fats or analogous substances present. On pursuing the investigation with regard to other tissues they found that the law held good. Thus we may take it that the "lipoid" element in blood and tissue cell probably forms a loose combination with the chloroform and that anaesthesia depends upon this. It is also notable that the heart muscle is found to have a much greater affinity for chloroform than skeletal muscle, as represented by the figures 41 mg. per cent. for cardiac and 21 mg. per cent. for skeletal muscle. Sherrington and Sowton (12) have shown that striped muscle - unlike cardiac - is not readily poisoned by chloroform. Thus the substances which fix the chloroform in cardiac muscle are present in smaller amount in skeletal muscle, and there is a lower susceptibility of the latter to the action of the drug.

The work of Brodie and Widdows (13) and Buckmaster and Gardner (7) agrees in all important respects with regard to the rapidity and rate of absorption. They show that chloroform absorption increases with great rapidity in the initial stages of anaesthesia to a value which approaches a maximum. Brodie and Widdows consider that the period of maximum absorption is during the second minute

minute of the administration, but Buckmaster and Gardner place the time somewhat later - from five to fifteen minutes in three instances cited. Following this maximum of absorption, the breathing becomes shallower, probably owing to the effect of the chloroform on the respiratory centres. This depression of respiration varies in different individuals, and appears to depend on the degree of concentration of the chloroform - air mixture. During the period of respiratory depression the rate of absorption diminishes.

At this stage in animals the authors found that there was danger of the respiration completely stopping, and that, although artificial respiration was frequently successful in restoring the breathing, this was not always the case. Buckmaster and Gardner state that all the deaths they had during this period were entirely due to respiratory failure, and none to cardiac failure. Levy (14) on the other hand, has shown that in 18 cases of sudden death in cats during light chloroform anaesthesia, fibrillation of the ventricles was the cause of death. This is referred to fully on page (65c). In any case the evidence is completely in agreement that a definite danger point occurs within the first few minutes of anaesthesia. This is represented



Curves showing rate of assumption of chloroform administered by inhalation. The rapid rise in amount per cent of blood in the early stage is accompanied by a period of danger shown between letters X and Y (Buckmaster, Gardner)

represented by the period between X and Y in the figure.

Sherrington and Miss Sowton (12) have shown that cardiac muscle is much more readily affected by chloroform solution plus CO_2 than by chloroform solution plus O_2, and consequently the period of diminished ventilation of the lung must necessarily be one of danger.

If the animal passes this stage, and the administration is continued, the absorption again increases, and the chloroform content of the blood rises again until a point of equilibrium is reached - intake and output going on side by side. This equilibrium may be maintained for a considerable time which varies in different individuals. The point of equilibrium is, however, not one of safety, for the difference between the amount of chloroform in the blood throughout this stage and that necessary to kill the animal is very small.

ELIMINATION OF CHLOROFORM

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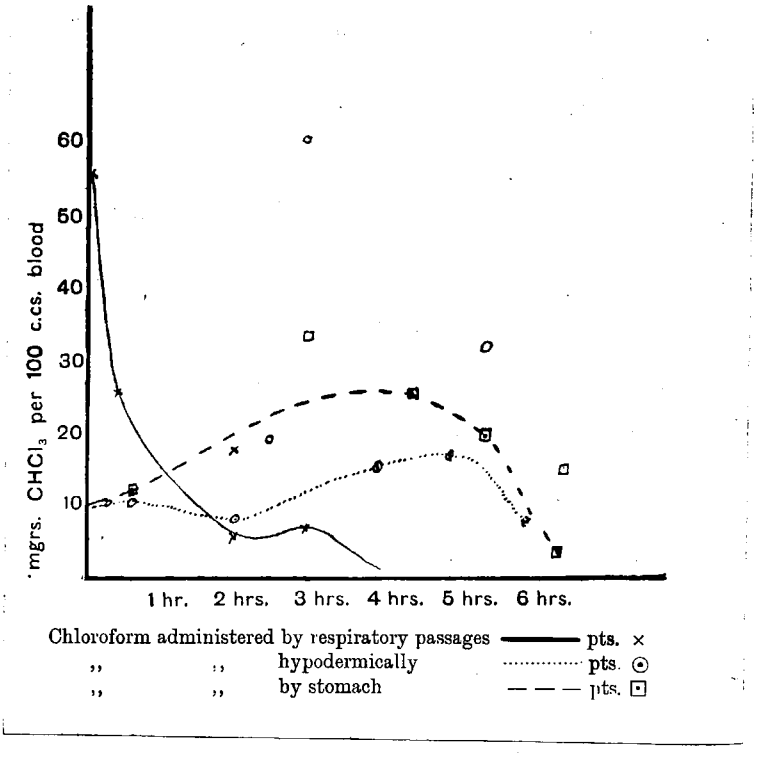
At the same time as absorption of the anaesthetic is going on, elimination is also taking place, and, as in the case of absorption, this does not take place at a uniform rate. The rate of elimination depends to some extent upon the condition of the animal under experiment, but generally it may be taken that the rate of output is rapid at first and slower after. When the point of equilibrium referred to above is reached, an administration of small amounts of the drug is all that is necessary to keep the animal anaesthetised - that is to say, both absorption and elimination are slow. The initial rate of elimination is slower than the initial rate of absorption, so that the blood is becoming more charged with chloroform.

After administration is stopped the rate of elimination is at first rapid; indeed according to Nicloux (15) half the chloroform content of the blood is eliminated in dogs in the first five minutes. Buckmaster and Gardner (7) found that half the chloroform content was eliminated in fifteen



Curves showing rates of assumption and elimination of Chloroform given in the three ways indicated

(Paton and Findlay)

fifteen to twenty minutes in cats, and in rabbits Paton and Lindsay (16) found that more than half the chloroform was eliminated in twenty five minutes. The remaining part was eliminated at varying rates. In some instances very little sign of chloroform was found after two hours, although in others about 20 mg. per cent was found - an amount equal to about one half of what is required to produce full anaesthesia.

After three hours a relatively large amount may still remain. Such instances may probably be considered abnormal, and that normally the elimination of chloroform given through the respiratory tract is comparatively rapid, particularly if the administration has been of short duration. Where elimination is delayed, however, the tissues remain bathed in blood containing chloroform for an extended period, and changes to be described shortly are produced in the tissues - notably in the liver.

The rates, both of assumption and of elimination, are very different when the drug is given in ways other than by inhalation. The figure shows the much slower rate of assumption and elimination when chloroform is given by the mouth or subcutaneously. The maximal amount in the blood

blood is not reached until about four hours after administration by the stomach, and not until about five hours after injection.

The observations of Paton and Miss Lindsay show that, in the rabbit, chloroform when given by the respiratory passages is rapidly taken up by the blood, and that anaesthesia is produced when the amount reaches about 30 to 40 mg. per 100 c.cm.

It is then rapidly eliminated, so that, in normal cases, by the end of two hours it has almost entirely disappeared from the blood. This elimination, however, is subject to marked variation, and at the end of two hours there may still remain in the blood no less than 20 mg. of the drug, and even after 3¼ hours 10 mgrs. may remain.

These experiments seem to explain the occurrence of late chloroform-poisoning in a certain number of cases where the drug remaining in the body has acted on the tissues for a prolonged period.

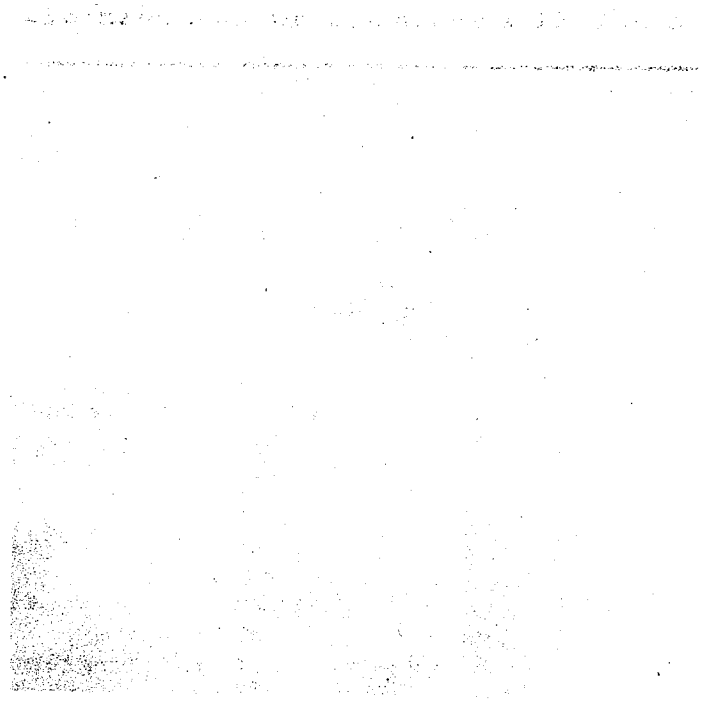
When given by the mouth, chloroform is slowly absorbed, the percentage amount reacning its maximum between four and five hours after administration, and then slowly disappearing. The amount taken up by the blood after the administration

administration of about 1 c.c. per 1000 grm. is rarely sufficient to produce anaesthesia - in only one case did it reach 32 mgrs., and in that case the animal was not anaesthetised, but merely staggery. In all probability, the fixation of the drug by the proteins and corpuscles prevented its full action on the nerve centres.

When chloroform is given hypodermically, absorptic is generally more rapid than when it is given by the mouth, and the percentage in the blood reaches its maximum in about four or five hours. The amount present is rarely sufficient to produce anaesthesia, but in one case it was produced when only 18.7 mgrs. were present. In one case when the respiration and heart stopped, no less than 60.9 mgrs. were found in the blood at the end of three hours; in another, at the end of 5½ hours 31.6 mgrs. were present, and yet no anaesthesia, but merely staggering, was observed

These observations show that the more marked action of chloroform on the metabolism when it is administered by the mouth or hypodermically is probably to be explained by the more prolonged action of the drug upon the protoplasm. The more marked action of the drug when administered by the mouth, as compared with the action of the same

same dose hypodermically administered, is probably due to the more direct action upon the liver cells, and possibly to its less rapid elimination, indicated by its more marked accumulation (see figure).



DISTRIBUTION OF CHLOROFORM IN THE BLOOD.

DISTRIBUTION OF CHLOROFORM IN THE BLOOD.

The Author was fortunate in obtaining the help of Miss Dorothy Lindsay in making estimations of the amount of chloroform present in the blood under various conditions.

Buckmaster and Gardner (17) have shewn that in chloroform anaesthesia the bulk of the chloroform is carried by the red corpuscles. Pohl (4) estimated the proportion in the corpuscles as two to four times that present in the serum. Nicloux (5) by a more accurate method estimated the amount in corpuscles as about 88 and in the plasma 12 per 100 parts of chloroform. The enormous difference in time of absorption and of elimination as shown above, suggests that the chloroform might be differently combined when it is inhaled than when it is injected or given by stomach. By inhalation the greater part of the chloroform combines loosely with the corpuscles and is rapidly absorbed and rapidly eliminated. A small moiety is sometimes found, according to Noel Paton and Miss Lindsay (16), still present in the blood some hours after the anaesthetic, particularly when the animal anaesthetised has been confined in a cage after the administration. This might represent

represent a remnant of the 12 per cent in the plasma, referred to on page 14 . When given subcutaneously the much slower absorption and elimination, and the consequent greater effect on the tissues, might be due to a firmer combination of the chloroform with some part of the plasma - say, the proteins, as suggested by Moore and Roaf ( ). That the drug is differently fixed in the two cases is further suggested by the fact that a prolonged administration of chloroform by inhalation has very little effect on the liver and kidney tissues, whereas the administration of a very small amount hypodermically causes injury to the cells of these organs.

In carrying out the experiments the same procedure was observed in all cases. The rabbits were weighed, and then where they were anaesthetised by inhalation they were kept just under for one hour. They were then killed and rapidly bled into an amount of 15 per cent. solution of potassium oxalate sufficient to prevent coagulation of the blood. The blood mixture was then centrifugalised for three hours, and the corpuscles and plasma were separately estimated for chloroform. The method of Nicloux (18) was used throughout the estimation.

The

The time taken for centrifugalising - three hours - is the same as was adopted by Nicloux, and yielded more constant results than any shorter time. As an objection against such a lengthy separation may be argued that both corpuscles and chloroform would be driven to the bottom of the tube, and thus the proportion in corpuscles would rise with prolonged separation. This, as a matter of fact, is the case, but may also be due to the separation being more complete. Where the centrifugalising has gone on for one hour, the corpuscles plus chloroform are mixed with a certain amount of plasma plus its chloroform; in the latter case the chloroform content is small and tends to reduce the proportion of chloroform in the mass. At the end of three hours the amount of plasma and chloroform with the corpuscles is very small indeed, and is negligible. In any case, the same procedure was adopted with both inhalation and injection specimens, so that the results are comparable.

Where chloroform was administered subcutaneously amounts varying from 1 c.c. to 3 c.c. were given, and the animals were killed and bled as above two to three hours later. The amount of chloroform in the blood approaches a maximum two to four hours after the administration. The results are:-

1. By Inhalation.

Rabbit D.- Weight, 1800 grm. Chloroform inhaled for one hour. 35 c.c. of blood taken with 1 c.c. of 15 per cent. potassium oxalate; centrifugalised for three hours. Total amount recovered, 18.13 mg. chloroform. Corpuscles 90.8 per cent; plasma, 9.2 per cent.,of total chloroform present.

Rabbit M.- Weight 2200 grm. Chloroform inhaled for 45 minutes. 58 c.c. of blood taken with 2 c.c. of 15 per cent. potassium oxalate; centrifugalised for three hours. Total amount recovered, 26.56 mg. chloroform. Corpuscles 90.2 per cent.; plasma, 9.8 per cent. of total chloroform present.

Rabbit P.- Weight, 1850 grm. Chloroform inhaled for one hour. 35 c.c. blood with 5 c.c. potassium oxalate; centrifugalised for three hours. Total amount recovered 6.3 mg. chloroform. Corpuscles, 88.8 per cent.; plasma, 11.2 per cent. of total chloroform present.

Rabbit R.- Weight, 1900 grm. Chloroform inhaled for one hour. 38 c.c. of blood with 5 c.c. of potassium oxalate; centrifugalised for two and a half hours. Total chloroform

chloroform recovered, 16.45 mg. Corpuscles, 88.9 per cent; plasma, 11.1 per cent of total chloroform present.

Rabbit S.- Weight, 2400 grm. Chloroform inhaled for one hour. 45 c.c. of blood with 5 c.c. of potassium oxalate; centrifugalised three hours. Total amount recovered, 14.84 mg. chloroform. Corpuscles, 87.6 per cent; plasma, 12.4 per cent. of total chloroform present.

Rabbit Z.- Weight, 1800 grm. Chloroform inhaled for one hour, 40 c.c. of blood, with 5 c.c. of potassium oxalate; centrifugalised for two hours. Total amount recovered, 14.84 mg. chloroform. Corpuscles, 85.2 per cent; plasma, 14.8 per cent of total chloroform present.

Average distribution in six rabbits; Corpuscles, 88.6 per cent; plasma, 11.4 per cent of total chloroform present.

2. By Injection.

Rabbit C.- Weight, 2400 grm, 1 c.c. injected; animal killed four hours later. 40 c.c. blood taken with 1 c.c. of potassium oxalate; centrifugalised three hours. Total amount recovered 7.9 mg. chloroform. Corpuscles, 81.6 per cent.; plasma, 18.4 per cent. of total chloroform recovered.

Rabbit G.- Weight, 2975 grm. 2 c.c. injected; animal killed two and a half hours later. 42 c.c. blood with 1 c.c potassium oxalate; centrifugalised for three hours. Total amount recovered 6.21 mg. chloroform. Corpuscles, 76.4 per cent; plasma, 23.6 per cent. of total chloroform present

Rabbit H.- Weight 1450 grm. 1.5 c.c. injected and repeated in 15 minutes. 30 c.c. blood and 1 c.c. potassium oxalate; animal killed two hours later; centrifugalised three hours. Total amount recovered, 8.25 mg. chloroform. Corpuscle, 78 per cent; plasma, 22 per cent of total chloroform present.

Rabbit Q.- Weight, 2300 grm. 3 c.c. injected and rabbit killed three hours later. 40 c.c. blood and 5 c.c. potassium oxalate; centrifugalised three hours. Total amount recovered, 5.88 mg. chloroform. Corpuscles, 74.8 per cent.; plasma, 25.2 per cent. of total chloroform present.

Rabbit T.- Weight, 2200 grm. 3 c.c. injected, 40 c.c. blood taken with 5 c.c. oxalate. Total amount recovered, 9.38 mg. chloroform. Corpuscles, 79.9 per cent.; plasma 20.1 per cent. of total chloroform present.

Rabbit Y.- Weight 2050 grm. 3 c.c. injected. Animal

Animal killed two hours later. 40 c.c. blood taken with 10 c.c. oxalate; centrifugalised three hours. Total amount recovered, 10.84 mg. chloroform. Corpuscles 72.4 per cent.; plasma, 27.6 per cent. of total chloroform present.

Average distribution in six rabbits:- Corpuscles, 77.2 per cent; plasma, 22.8 per cent. of total chloroform present.

Consideration of Results.

Where the chloroform was inhaled the amount found in the plasma varied from 9.2 to 14.8 per cent. of the total amount recovered from the blood. Where the chloroform was injected the amount found in the plasma varied from 18.4 to 27.6 per cent. of the total amount recovered from the blood. Thus in all cases where the chloroform was given subcutaneously the amount recovered from the plasma was much more than where the anaesthetic was inhaled. The delayed elimination therefore appears to be due to the different fixation of the drug in these cases.

It is of interest to note that in one rabbit which died just before the chloroform inhalation was stopped - after one hour's inhalation - the amount of chloroform recovered was 22.24 mg. in 35 c.c. of blood, and of this the corpuscles

corpuscles contained 67.7 per cent. and the plasma 32.3 per cent., so that the fatal result was at least associated with a large percentage of chloroform in the plasma.

INFLUENCE ON THE TISSUES OF A SINGLE ADMINISTRATION.

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Before making observations upon the histological changes following administration of chloroform, a series of experiments were conducted, in which small blocks of tissue were immersed for varying times in saline solutions containing chloroform. The results may be summarised thus: When kidney or liver tissue is immersed in a saline solution containing chloroform, degenerative changes take place similar to the normal necrobiotic changes, but are very much more rapid. In the case of the kidney the glomeruli are not affected for a very considerable time.

1. Administration by Inhalation. Assumption and elimination of the drug are both rapid in this method of administration, and consequently the drug is acting for only a short space of time upon the tissues examined.

In the tissues taken from animals which have had chloroform administered through the lungs, the amount of change in the liver and kidney - which were the organs carefully examined - was on the whole but small.* In some cases

* Photomicrographs of specimens are shown in Appendix I., page 83

cases, notably those examined some time after anaesthesia, no variation from the normal was observable. In one rabbit which was killed immediately after the administration, the cells lining the descending and ascending tubules of Henle and the first convoluted tubules showed degenerative changes. The liver was less affected, the cells being in an early stage of albuminous degeneration. The cells of the liver showed granules which did not react to the stains for fat.

At the time the animal died, the blood contained 77.3 mg. of chloroform per 100 c.c. of blood. Respiration had stopped during the administration of the anaesthetic. In a small number of other animals experimented on, changes were observed in kidney and liver.

When chloroform is administered through the respiratory passages, a considerable amount of degeneration is only occasionally found in the kidney and liver cells. It is more marked in some cases than in others where a similar amount of chloroform was given to animals of similar size. This may be associated with the varying rate at which the drug is eliminated as shown by Miss Lindsay (16). The degree of change in the liver was never great. In the kidney there is frequently cloudy swelling and occasionally desquamation

desquamation of the epithelium of the tubules.

2. Administration by the Stomach. In this series of observations 1.c.c. of chloroform mixed with 9 c.c. of olive oil was introduced during light anaesthesia into the stomach of rabbits. The weights of the animals varied between 1000 and 2300 grms.. The mortality was very great. The animals which survived the experiment were killed at different periods after the administration of the drug. In the organs examined the degree of change was found to vary greatly with the length of time after the chloroform was administered. In cases where the animal was killed within a few hours of the administration very little change was observed in the kidney and liver - the organs examined.

After three hours, the cells of the convoluted tubules of the kidney showed marked cloudy swelling. Here and there desquamation was seen in the ascending and descending tubules of Henle. In the liver the cells at the periphery of the lobules showed but little change - at most a slight degree of cloudy swelling. The cells in the centre of the lobule, on the other hand, had undergone a granular change, and the nuclei had begun to lose their power of taking on the stain. The granules gave a negative reaction to the stains for fat.

After

After five hours the cells lining the tubules of the kidney now showed marked degeneration and the tubules contained albuminous material. The cells were frequently vacuolated and the nuclei stained badly.

In the liver, the degeneration of the central cells of the lobules was more marked, and the granules now stained red with Scharlach Rot. The peripheral cells were apparently healthy.

At longer periods after the administration the degeneration was found to be more advanced, and in animals killed when obviously dying the kidney was found to be rapidly losing all signs of its original structure. The glomeruli were, however, affected only slightly, except in the animals which suffered most from the administration. Haemorrhages in the kidneys were frequently observed, and in such instances dark coloured granules, similar to those described by Fraenkel (19) Marthen (20) and Cohn (21) were generally seen in the cells lining the tubules adjacent to the blood (figures Appendix I. In the liver further changes resulted in the complete breakdown of all the liver tissue into granular debris, excepting a layer two or three cells thick at the periphery of each lobule. In the granular

granular debris granules reacting to the stains for fat were seen.

3. Administration by subcutaneous injection. As in case of administration of 1 c.c. of chloroform by the stomach, the injection of 1 c.c. resulted in a high mortality in the animals experimented upon.

Histologically the changes in the organs are very similar to those detailed above, the difference being one of degree.

In the kidney, four hours after the injection, very little degeneration was observed, only a slight degree of cloudy swelling being apparent. After five hours, some vacuolation was seen in one of the specimens examined. In one animal killed some two hours later, and in a dying condition, (fig. Appendix) the kidney showed an appearance comparable with that observed in some of the worst cases after administration by the stomach. The convoluted, the ascending and descending, and the collecting tubules showed little sign of cellular lining. They were choked with albuminous debris. The glomeruli retained an appearance approximateing to the normal. There was generally congestion and haemorrhage in the kidneys examined.

In

In the liver there was generally some necrosis in the centre of the lobule even a very few hours after the administration, and as time advanced this increased in amount (figure). In the worst cases where the animal was killed when dying, the organ was a honeycomb of cheesy material showing very little sign of the original liver structure.

It is notable, however, that the effect of the chloroform on the liver is not so marked when it is injected as when it is given by the stomach.

INFLUENCE ON METABOLISM OF A SINGLE ADMINISTRATION.

INFLUENCE ON METABOLISM OF A SINGLE ADMINISTRATION.

A substance exerting such poisonous effects upon the tissues as those detailed above might be expected to influence profoundly the metabolic processes of the body, That chloroform has such effects has been abundantly shown by Salkowski (22) and other observers. The ~~most~~ recent work by Noël Paton (23) illustrates the points well with regard to the three methods of administration and may be alluded to at some length.

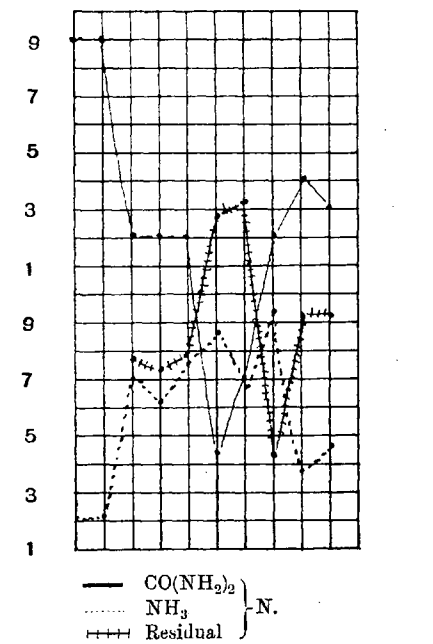
The effects of the drug upon metabolism are deduced from the changes in the products of protein breakdown as found in the urine. Any quantitative changes are indicated by changes in the total amount of nitrogen excreted per diem, while qualitative changes are indicated by alterations in the distribution of nitrogen and sulphur in the different nitrogen- and sulphur-containing constituents of the urine. In the experiments performed the animals were kept upon a fixed diet until metabolic equilibrium was established, the drug was then given, and the amounts of nitrogen and sulphur in the various constituents of the urine

urine were then investigated for a longer or shorter period depending upon the degree of disturbance which resulted.

The results of these observations may be briefly summarised. When chloroform is administered through the respiratory passages, the effect upon metabolism is, at worst, but slight. The hepatic metabolism, by which ammonia compounds are changed to urea, appears to be stimulated. This stimulation does not seem to be followed by any after-depression. There was occasionally an increased conversion of ammonia to urea. Thus the proportion of Nitrogen as urea remained unchanged or slightly increased, while the Nitrogen in NH_3 was unchanged or slightly decreased.

Noël Paton shows that in a few instances the drug exerts a toxic action upon the kidneys. This is demonstrated by the existence of porteins and of renal epithelium in the urine.

When given by the stomach the protein disintegration caused by the administration of chloroform is greatly increased, particularly on the day of or the day after the administration. After a day or two the proportion of the various Nitrogen-containing substances in the urine undergo marked changes, the Urea - Nitrogen diminishes



Curves showing the distribution of the urinary nitrogen after Chloroform administered by the stomach (Paton)

diminishes, the NH_3 - N increases, and later the residual N increases (see Fig.X.). The increase in the latter is very marked and Noël Paton suggests that it probably represents the appearance of amino-acids. The proportion of unoxidised sulphur to total sulphur shows a distinct fall.

With the idea of throwing some light upon the nature of the products forming the residual N, and thus testing Paton's hypothesis as regards the presence of amino-acids, Miss Lindsay (24) made complete analyses in the case of three dogs. She found, in addition to the increase in total nitrogen and the diminution in urea-nitrogen, with rise in NH_3 nitrogen, that the residual N could be divided into allantoin N, amino acid N, creatinin N, and creatin N with a small percentage of undetermined nitrogen.

A slight rise in allantoin-N is noted, which is probably caused by the toxic action of the drug upon the liver cells.

The amino acid-N rose in each case, the rise generally being in mon-amino acids, but in one case the rise was probably due to increase in di-amino acids and polypeptides. The creatinin-N remained practically unchanged, but creatin appeared in the urine two or three days after the administrations. This disturbance of the creatin metabolism

metabolism is associated with the disturbance in the hepatic functions, and Underhill and Kleiner (25) found fatty degeneration and creatin excretion following injection of hydrogen sulphate, while Mellanby (26) found creatin in considerable amount in cases of carcinoma of the liver. The amount of creatin in the urine may be taken, according to Hoogenhuyze and Verploegh (27), as an indication of the extent of the hepatic disturbance.

The undetermined nitrogen probably represents diamino acids and polypeptides.

These effects upon the metabolism suggest that when given by the mouth chloroform exerts a toxic action upon the liver cells, notably, a day or two after the administration. The urine also shows the presence of tube-casts, renal epithelium and protein, so that the kidney is affected also. This is much less marked when chloroform is given as an inhalation.

When chloroform is given hypodermically it acts in the same way as when given by the mouth, but not so markedly. The toxic effect upon the liver cells does not appear to be so great as when the drug is given by the mouth. There may be a fall in urine N, with a rise in residual N and in ammonia N, but in other instances there may be

be no definite change in the nitrogen excretion.

Thus it would seem as if the rapid assumption of chloroform, when administered through the lungs, and the relatively rapid elimination, stimulate the hepatic metabolism; whereas the chloroform, more slowly absorbed and eliminated, and therefore having a much more prolonged action when given by the mouth or hypodermically, leads to an actual injury of the liver and kidney cells.

THE INFLUENCE OF CHLOROFORM UPON THE BLOOD.

THE INFLUENCE OF CHLOROFORM UPON THE BLOOD

As chloroform is carried to the tissues by the lipoids of the blood, the author considered it a matter of importance to investigate the question whether the blood corpuscles were effected by the combination of chloroform with their lipoids. Any effect upon the corpuscles might show themselves on Histological examination or in blood counts taken at various times after the administration.

In his examination of the blood after chloroform, Dawson made several red blood counts in addition to many leucocyte estimations, and arrived at the conclusion that there was practically no variation in the number of red corpuscles, although he refers to a slight but persistent rise after administration of chloroform to a dog. As the blood counts were made in most cases for only some hours after the anaesthetic, and as in any case it is improbable that much variation could take place in that time, a series of counts was undertaken to endeavour to find what effect the administration of chloroform had upon the blood corpuscles as shown on the days following the administration.

The

The leucocyte counts have been fully dealt with by Dawson, who found a slight diminution after chloroform anaesthesia. (28)

As in the previous sections of this work, the chloroform was administered in one of three different ways (a) through the respiratory passages, (b) by subcutaneous injection and (c) introduced, mixed with olive oil, into the stomach by means of an oesophageal tube. The method of estimation adopted was the Haemocytometer of Thoma as made by Zeiss. Two sets of 3 large squares - 96 small squares were counted for each estimation.

In the first place a series of control estimations were made with normal rabbits and the variation from day to day was found to be very little over 5% rise or fall. For example rabbit 7 gives for six consecutive days.

6,480,000, 6,160,000, 5,800,000, 5,840,000, 6,000,000
6,300,000

and No.53 for a similar period gave

5,760,000, 5,430,000, 5,360,000, 5,200,000,5,340,000, 5,200,000

Blood counts were now carried out in animals before and after the administration of the drug. In some instances pre-chloroform blood-counts were continued for some

some days before the drug was given so that the normal variation was known.

Administration by Inhalation.

The degree of change in number of red corpuscles varied to some extent with the length of time during which the anaesthetic was administered, but the variation was not constant. In all cases the chloroform was administered for ½ or 1 hour and for one time only.

Table I. shows, in millions, the variation in number of red corpuscles from day to day after the administration.

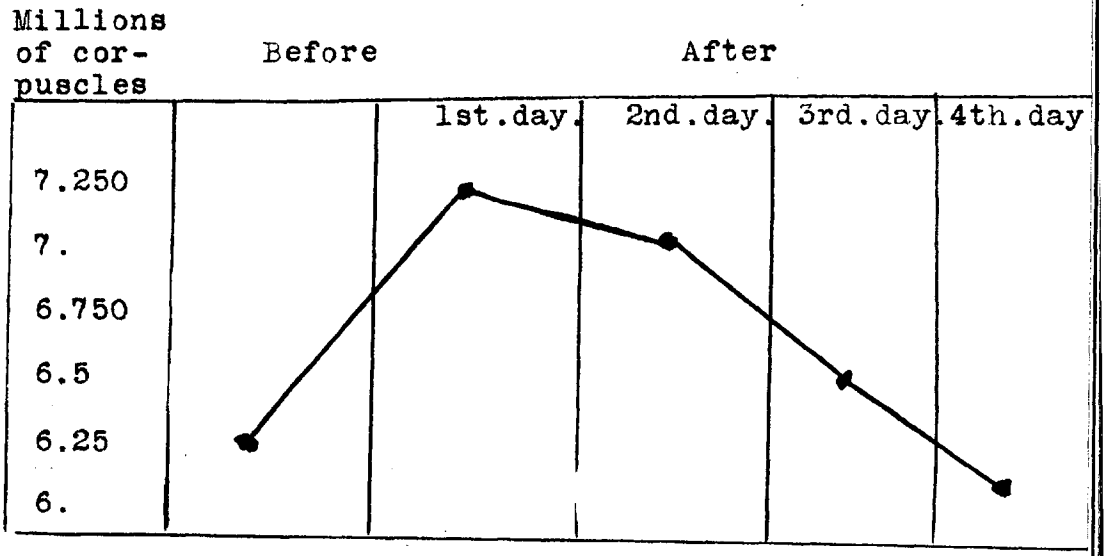
No. of Animal.	Before administration	Day after	2nd. day after	3rd. day after	4th. day after	5th. day after	6th. day after	Rise per cent	Time of administration
19	6.9	8.24	8.4	7.48	7.36	7.6	6.9	20	½ hr.
20	5.84	6.56	7.20	6.40	5.60			12-20	½ hr.
22	6.08	7.44	7.56	6.6	5.6			24	1 hr.
26	6.0	7.28	7.24	7.62	6.32	6.44	6.9	20	½ hr.
32	5.44	4.25	4.72	4.4	4.83	died			1 hr
35	5.32	6.5	6.34	5.43	5.47			24	½ hr.
37	6.23	7.03	6.66	6.06	6.93			16	1 hr.
38	7.09	7.73	6.67	7.12				10	1 hr.
39	7.36	6.42	4.87	4.31	died				1 hr.
40	5.76	6.49	6.59					13	1 hr.
41	6.8	7.74	6.76	6.24	5.92			11	1 hr.
43	6.75	7.44	6.94					10	½ hr.

Average rise in the 10 animals that survived 16%

It will be seen from this table that two rabbits showed a diminution in the red blood-counts after the anaesthetic and both of these animals died, one on the third day and the other on the 4th.day after the anaesthetic.

On the other hand in the ten cases where the animal survived for a week at least, or which were killed while apparently healthy, the red blood corpuscles increased in number. The smallest increase was in Nos.38 and 42 where the corpuscles increased to the extent of 10% in each case. In Nos.22 and 35 on the other hand, the increase was as much as 24%.

For the whole series the results may be mapped out in the form of a curve thus:-



The

The rise is a sharp one and in a few cases the crest of the wave is only reached on the second day after the anaesthetic.

Administration by Subcutaneous Injection.

Here the change in red blood-counts was very marked - much more so than in the case of administration by inhalation.

In all cases one or two counts were made and then 1 c.c. of pure chloroform was injected subcutaneously.

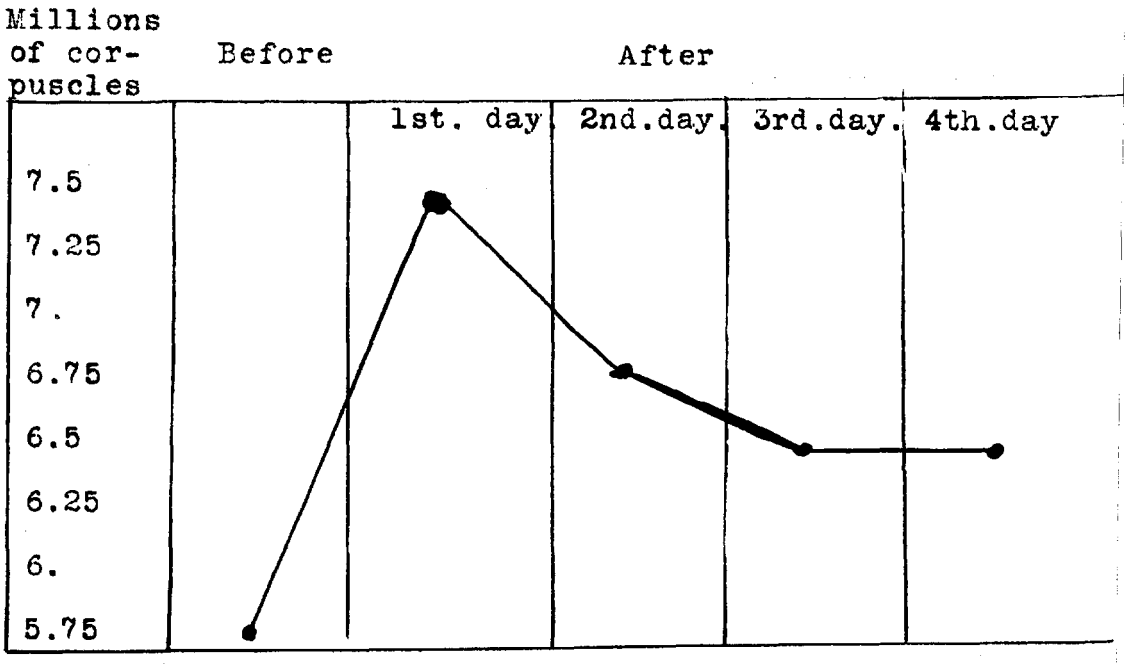
Table II. shows, in millions, the variation in number of red corpuscles from day to day after the injection.

No.	Day of injection	1st. day after	2nd. day after	3rd. day after	4th. day after	5th. day after	6th. day after	Rise %
5	6.32	7.5	6.6	6.4	6.8	6.3		19
6	5.6	4.8	died					fall
8	5.6	7.84	7.6	6.68	6.4	6.6	6.3	25
9	5.6	6.7	5.85	6.16	5.84	5.4		20
10	5.6	5.6	died					
12	5.76	6.5	6.72	5.84	6.0	5.6	6.92	14
13	5.04	5.12	5.48	died				1.5
14	5.4	6.96	6.64	6.08	6.4			29
15	5.4	6.48	6.64	6.5				20
21	5.6	7.44	7.2	7.04				33
24	6.32	8.88	killed					40
42	6.79	8.20	7.23	7.44	7.52			21
							Average	25.5

The rise in number of red cells is very striking, and it is of considerable interest that in the three cases where there was either a fall in the count or an inconsiderable rise, the animal died within a very short time.

The average rise for the day after the administration is 25.5% in the 9 experiments cited, compared with 16% in the 10 experiments where chloroform was inhaled.

For this series the curve is as under.



At the end of the 4th.day after the administration the normal had not been reached in most of the cases. This generally was not arrived at until the 8th.or 10th. day.

The

The crest of the wave was reached on the day following the injection of chloroform in all the experiments performed in this way.

Administration by the Stomach.

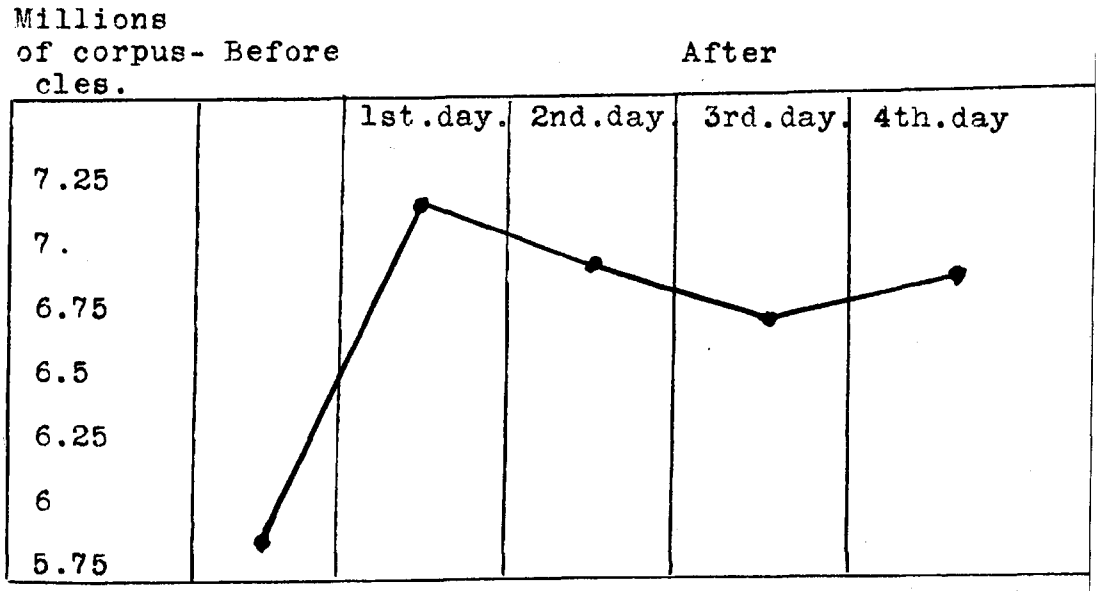
One cubic centimeter of chloroform was made up to 10 c.cs. with olive oil and administered through an oesophageal tube passed while the rabbit was under ether anaesthesia. The question of the effect of such a dose of pure oil was first considered, and that amount was administered in 3 experiments without an appreciable rise on the following nor on the third day.

The results when chloroform was given were as follows.

No.	Day before	1st.day after	2nd.day after	3rd.day after	4th.day after	5th.day after	6th.day after	% Rise
33	5.46	7.3	6.16	6.02	6.07	5.56	killed	35
36	5.84	6.72	6.48	5.88	5.7	killed		15
44	7.60	8.8	7.6	died				16
45	6.4	7.94	7.28	7.46	7.2	6.4	6.24	24
46	6.16	7.82	died					28
53	5.2	4.8	4.52	died				fall
51	7.36	7.28	died					fall
56	5.84	7.6	8.45	8.24	7.67	killed		31
57	6.06	6.4	6.4	6.32		killed		7

Average Rise 22%

The mortality in rabbits that have chloroform administered in this way is always high and it is most significant that in both cases where the red-count diminished the animal died, comparing in that particular with those animals which had the anaesthetic administered in the orthodox way, through the respiratory passages, and with those where the drug was injected subcutaneously. In those animals which survived the administration the curve is as follows.



The average rise for the day following the administration is 22%. nearly as high as in the experiments where chloroform was injected subcutaneously.

Microscopical Appearance of the Blood.

The change in the number of the red corpuscles was so marked and so uniform that films were made and examined to see if any signs could be found of unusual activity in the bone marrow to account for the polycythaenia. Specimens were taken the day before the anaesthetic was administered and then for several days afterwards. The stain used throughout was Jenner's stain or Leishman's, occasionally made up from tablets, but generally from powder.

The first blood was taken from rabbit No.14, and examined with a Zeiss $\frac{1}{12}$ homogeneous immersion objective. About 2 per cent of the red corpuscles were found to be in a state of granular degeneration. The granular degeneration was very marked and was observed for some days afterwards, the number of granular cells becoming reduced as time progressed. The observations were repeated with rabbit No.15 and again the degenerated cells were found. As these cells were not found in films made before the administration, it appeared obvious that they resulted from the administration. In all a series of 10 rabbits with the anaesthetic administered in the three different ways quoted above

above showed definite granular degeneration after administration of the drug.

Microcytes and poikilocytes were of frequent occurrence in all films examined after chloroform, but no nucleated red cells were found.

Examination of the Bone Marrow.

In a large proportion of cases the bone marrow was examined. Signs of activity were present in most of the specimens examined, and in a few instances the activity was very marked indeed. On the other hand in some of the rabbits that died there was evidence of hyaline degeneration.

Consideration of Results.

The very marked polycythaenia that is referred to above requires some explanation, and there are several possible causes for this phenomenon.

In the first place Nothnagel and Ostertag held that the action of chloroform was primarily upon the red corpuscles, and that these were in part destroyed. This conception

conception is to some extent supported by Buckmaster and Gardner (17) who showed that during the earliest stages of anaesthesia the blood became rapidly charged with chloroform which is held chiefly by the red corpuscles. Those red corpuscles which hold the chloroform must be to some extent affected by it and might quite well be the degenerated cells seen under the microscope.

The presence of a number of effete corpuscles in the blood which are unable to carry on their usual function would suffice to stimulate the bone marrow to activity. The chloroform itself, which is lethal after a time, primarily acts as an exciting agent on living cells, and would therefore stimulate the marrow cells to activity.

Another possible explanation of the increased number of red cells might be that there is an increased output of urine during or after anaesthesia. According to Thompson(19) who conducted a series of experiments on dogs, which were anaesthetised by inhalation for periods of from two to four hours, the quantity of urine secreted increases during the early stages when the anaesthesia is light, but is diminished or suppressed during full anaesthesia.

During chloroform anaesthesia the urine secreted is

is invariably more dilute than normal, even with diminished amount. He finds that there is a general but not accurate correspondence between urine outflow, kidney volume, and blood pressure. After the removal of the anaesthetic the amount of urine secreted increases greatly, sometimes as much as four times the normal. The maximum outflow occurs about three hours after removal of the anaesthetic.

It seems probable, then, that the increased number of red corpuscles is due in part at least to increased concentration of the blood.

In an effort to prove this point, the author took normal rabbits and estimated the specific gravity of their blood. Other rabbits were taken after administration of chloroform, and the specific gravity of their blood was estimated. The results of the experiments are recorded in Appendix III , and are in favour of this last hypothesis.

THE EFFECTS OF CHLOROFORM WHEN REPEATEDLY ADMINISTERED IN SMALL DOSES.

THE EFFECTS OF CHLOROFORM WHEN REPEATEDLY ADMINISTERED IN SMALL DOSES.

The very striking results obtained when administering a single large dose of chloroform and referred to at length above, suggested to the author that a very much smaller dose might have some influence on the tissues of an animal, particularly when the small dose was repeated daily.

Sir James Crichton-Browne in 1907 ( ) called attention to the habit in certain districts of taking lozenges and jujubes containing chloroform, and pointed out that samples of the lozenges contained as much as 2.9 per cent. of chloroform. He pointed out that these lozenges are taken in considerable quantity and over long periods, and he expressed the opinion that such repeated doses must be harmful. The repeated administration of small doses of this drug by the stomach to lower animals is therefore of practical as well as of scientific interest.

In the series of experiments undertaken rabbits were used throughout and the chloroform was administered - either

either by inhalation, by the mouth, or subcutaneously. The weight of the animal was taken day by day, and after death in many cases the weight of the heart was taken, and also the weight of the spleen. In all cases portions of liver, spleen, kidney, and heart were examined for fat and also stained and examined for degenerative changes. In the first experiments of the series the animals which were injected with $CHCl_3$ had 0.2 c.c. on two consecutive days, and two days were allowed to elapse before this procedure was repeated. This method proved so rapidly fatal that in the subsequent experiments 0.1 c.c. of $CHCl_3$ was injected daily. In the cases where the rabbits received the $CHCl_3$ in the form of an inhalation they were rapidly anaesthetised and then kept lightly under for 15 minutes. In the first experiments of this series this was done daily, but as all the animals used died while under the anaesthetic after a very few days, the procedure was modified, and the anaesthetic given on alternate days. When $CHCl_3$ was given by the stomach it was found that $CHCl_3$ water was easily tolerated by the rabbits and consequently 40 c.c. of this solution were given daily. This contained 0.08 gramme of chloroform. In some cases the rabbits lapped up the fluid without trouble, but in most instances

instances a stomach tube was passed under light ether anaesthesia and the $CHCl_3$ water administered through this. Considering the results of the experiments throughout the series the effect of the $CHCl_3$ administered in the various ways upon the well-being of the animal may be studied by an examination of the weights from day to day. The weights are given in grammes.

Table I. Administration by Inhalation

No.	Day									
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.
6	1470	1450	-	-	-	-	-	-	-	-
7	950	920	860	770	-	-	-	-	-	-
8	2250	2250	2120	-	-	-	-	-	-	-
12	1850	1725	1750	1780	1880	1855	1850	1870	1855	1750
20	2300	2275	2270	2270	2250	2200	2400	-	-	-
22	2050	1975	1950	1900	1850	1850	1800	1750	1700	1625*
24	2250	2170	2250	2150	2250	2250	2100	2150	-	-
26	1600	1600	1700	1750	1750	1750	1700	-	-	-
27	1650	1600	1550	1700	1750	-	-	-	-	-
30	2050	1950	1800	1670	1600	-	-	-	-	-
32	2370	2270	2260	2250	2300	2100	2050	1950	1925	1950+
36	1600	1550	1550	1550	1600	1600	-	-	-	-

* Survived for one month. Weight at death 1400 grammes

\+ Survived for two months. Weight at death 1840 grammes.

Where the administration took place daily the weight fell fairly rapidly. In the case of rabbit No.7 the weight had fallen nearly 200 grammes in four days. Where the administration was upon alternate days there was in most cases but little change. The daily rise or fall was similar to what was observed in control rabbits. In a few cases, however, a marked fall in weight took place - e.g., rabbits Nos.22, 30, and 32. The rabbits took food well, and some lived for a long while, having the chloroform administered as described - e.g., No.22 lived for more than a month and No.32 for nearly two months.

Table II. Administration by Subcutaneous Injection.

No.	Day											
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th
1	1150	1150	1100	1050	1070	1075	1100	1050	1070	1000	-	-
2	2000	1950	1890	1925	1900	-	-	-	-	-	-	-
3	1550	1550	1520	1470	1550	1450	1430	1450	-	-	-	-
	Daily Injections of 0.1 c.c.											
4	1700	1680	-	-	-	-	-	-	-	-	-	-
5	1430	1400	-	-	-	-	-	-	-	-	-	-
13	2400	2450	2430	2230	-	-	-	-	-	-	-	-
16	2700	2630	2520	2625	2500	2470	2400	2310	2220	2180	2150	2130
19	1700	1680	1700	-	-	-	-	-	-	-	-	-
21	3100	3050	3050	3000	3050	3020	2975	2930	2900	2850	1mnth	2725
25	1650	1550	1470	1450	1450	1350	1300	1350	1300	1375	1350	1250
28	1500	1400	-	-	-	-	-	-	-	-	-	-
29	1150	1150	1150	1150	1100	1060	-	-	-	-	-	-
31	2450	2420	2280	2250	2350	2450	2300	2150	2100	1950	26days	1350
37	1325	1300	1200	1100	1200	1200	1150	-	-	-	-	-

In the first three experiments cited above 0.2 c.c. of the anaesthetic was injected on the days signified by italic figures, and it will be noted that the effect of the injections is to lower the weight on the day or days following. This effect is very much more marked in the nine experiments of the second series when the chloroform was given daily until the animal died. Here a considerable fall in weight is shown in some of the animals. In rabbit No.21 there is a loss of 720 grammes in a month, and in rabbit No.21 a loss of 1100 grammes in a somewhat shorter time. In many of the animals the loss in weight is very slow at first, but as time progresses it becomes much more rapid. The effect upon weight of administering the chloroform in this way is much more marked than when the drug is administered by inhalation on alternate days, but not so rapidly fatal as where it is given daily in that manner.

Table III, Administration by the Stomach.

No.	Day											
	1st.	2nd.	3nd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.
9	2380	2320	2200	2250	2250	2160	2210	2175	-	-	-	-
14	2300	2350	2380	2280	2240	-	-	-	-	-	-	-
15	2150	2025	1975	2050	2150	1950	1925	1900	1940	1875	1870	1870
18	2400	2520	2500	2550	2450	2300	2150	4months later 1350				
23	1700	1700	1750	1700	1650	1550	1500	1500	1450	-	1mnth	1620
33	2100	2000	2000	1900	1900	1800	1700	1650	1600	1550	1475	-

The amount of $CHCl_3$ given by this method was somewhat smaller than that given in the two previous series, being about 0.08 gramme per diem, but this amount, small as it is (about the same as the $CHCl_3$ content of one lozenge as described by Crichton-Browne (3), caused a progressive diminution in weight in the animals. In all these cases the diminution in weight was marked, with the exception of No.14, where a rise in weight was found. The death of the animal was, however, preceded by a very considerable fall. In No.18 the rabbit tolerated a daily dose for over four months, but it is significant that in that time the animal had lost nearly 50 per cent of its original weight - or rather more than a kilogramme.

Histological Changes.

Concurrently with the alteration in body weight in the animals under experiment, histological changes in the organs and tissues examined were found. These changes were for the most part similar to those already described by Bandler (30), Heintz (31), and Ajello (32), and later by Doyon (33) and Billet (34).

Administration

Administration by Inhalation.

As in the previous series of experiments (p2) the kidney and liver suffered considerably when chloroform was administered in this way. The degree of degeneration, however, varied in the different cases, and the difference in degree was not always dependent upon the number of administrations, nor was the change equally marked in the kidney and liver. For example, in the kidney the greatest alteration from the normal was found in animals No.22, which survived for 31 days, and No.36, which only survived for six days. The liver in No.22 was also very badly degenerated, whereas that of No.36 was not markedly changed. On the other hand, the liver in rabbit No.30, which lived for five days, showed marked degenerative changes. The cells were coarsely granular and vacuolated; the nuclei took the basic stain badly and fat was abundant. On the whole, it may be stated that the degeneration in the kidney was the most marked feature of administration in this way, and that the kidney was invariably the seat of much change. A point of interest lies in the fact that most of the specimens showed a phenomenon which I showed in 1908 was only present after very great changes had taken place in the organ. The liver suffered

suffered most in those cases which survived for a considerable time, whereas those animals which succumbed at an early date showed generally a much smaller degree of change. Fat was detected in all instances, the stain used being Scharlach R. The fat was almost always abundant, and generally involved the cells at the centre of the lobule first and extended outwards subsequently.

The spleens of all the animals were examined and the sinuses were invariably found to be engorged with blood. A considerable quantity of an orange-coloured pigment was generally present, and in most instances enormous phagocytes were abundant, the phagocytes being distended with red corpuscles and often with pigment. The weight of the spleen varied considerably. Four control animals yielded an average weight of 0.78 gramme, each of the animals weighing 2000 grammes or over.

Table IV.

Table IV. Weight of the Spleen in Animals Anaesthetised.

No.	Original weight of animal.	No.of days.	Weight of Spleen
12	1850 grammes	20	2 grammes
20	2300 "	7	0.9 grammes
22	2050 "	31	1.2 grammes
24	2250 "	8	1.95 grammes
26	1600 "	7	0.6 grammes
27	1650 "	5	1.4 grammes
30	2050 "	5	0.72 grammes
32	2370 "	65	1.2 grammes
36	1600 "	6	1.2 grammes

The average weight of the animals was 1900 grammes while the average weight of the spleens was 1.24. A large increase of iron, as demonstrated by the relative amount shown by the ferrocyanide HCl stain on the sections, was found in all cases.

In examining the animals after death it was frequently found that the heart muscle appeared flabby and often the walls seemed unusually thin. These organs were therefore fixed and examined for histological changes. The heart muscle stained unequally; some parts appeared quite normal, while other areas showed a granular appearance quite unlike the normal; the transverse striations were lost and the fibres were often segmented. The nuclei in these parts were poorly stained and in some specimens little was left but

but a mere indication of the position which they occupied. A large number of the cells were vacuolated. This appearance is referred to by Ungar and Junkers (35), and by Heintz (34). The granular and broken-down appearance was very marked in some of the hearts examined, notably in Nos.12, 20, 22 and 24, but not so marked in No.32, where the animal survived for 65 days.

It may be noted here that all the animals referred to above died while ,under the anaesthetic, either while the drug was being administered or within five minutes after stopping the administration.

Administration by Subcutaneous Injection.

In the case of animals Nos.1, 2 and 3 chloroform was given in doses of 0.2 of a cubic centimetre, and the doses were given irregularly, as shown in Table II. The results in these cases are therefore not strictly comparable with those obtained by daily administration of 0.1 c.c., but they are included here as they illustrate the points to be brought out in this thesis.

The animals referred to other than the above received 0.1 c.c. hypodermically each day. The injection appeared

appeared to have no immediate effect, and the rabbits so treated were as active after as they were before the administration. Of those which were injected with the larger amount one died after four and two after three administrations, so that the drug was rapidly fatal. Those rabbits which were injected with only 0.1 c.c. lived for longer periods, the average being 11 days. The longest period a rabbit lived during the administration was 30 days and the shortest two days.

The organ which appeared most affected histologically was the liver, and here the changes were comparable with those referred to on page [illegible]. In one instance, where the rabbit survived for 30 days, the liver was found to be of an orange colour, and contained masses of an orange or yellow coloured pigment like that observed in the spleen, and similar to that described by Fraenkel (36), Marthen (37) and Cohn (38). This pigment gave a positive stain for iron. There did not appear to be atrophy. In a few of the livers examined, particularly when taken from animals which had lived for some time, a considerable amount of white fibrous tissue was found between the lobules, as described by Fiessinger (39). The kidneys also generally showed some degree

degree of degenerative change, although in many instances but little alteration from the normal was observed. Congestion and haemorrhage were generally found, and in the kidneys taken from the animals which were injected with the larger dose of the drug the degeneration in the convoluted as well as the straight tubules was very marked. Frequently the tubules were packed with debris and desquamated epithelium. Where the degeneration was far advanced fat was always found in the degenerated cells. On the whole, however, the kidneys appeared to be affected to a somewhat slighter degree in this method of administration.

The spleens showed much the same condition as did those in Table IV. The amount of iron present as shown by the ferrocyanide HCl method of staining was very great, and in No.19 when the animal was killed after three administrations fully half of the spleen section stained green by this method of staining. The large phagocytes referred to above were also present here in large numbers.

Table V.

Table V. The Weights of the Spleens and other Particulars.

No.	Original weight of Animal.	No.of days.	Weight of spleen.
16	2700 grammes	13	1.5 grammes
21	3100 "	30	1.85 "
25	1650 "	15	0.67 gramme
28	1500 "	3	1.2 grammes
31	2450 "	26	1.5 "
37	1325 "	7	1.5 "
Average	2121 "	16	1.37 "

Thus the average weight of the spleens was 1.37 grammes, as compared with an average weight of 0.78 gramme in the control animals. The hearts generally showed degenerative change, although this was not always marked. Granular cells with loss of the cross-striped appearance were common, and in most cases the nuclei stained irregularly; sometimes the peripheral part of the cardiac muscle appeared to have suffered most, at other times the central parts.

Fat was not observed in any of the hearts examined for it, therein differing from the findings of Fraenkel (36) and supporting Heintz (?).

Administration by the Stomach.

The method of administration adopted here was not by

by any means so rapidly fatal as either of the other methods, contrasting in this with the administration of larger doses in olive oil as detailed on page , where this was the most fatal method of administration. The average length of life during the administration was 16 days, not taking into account the animal that lived for four and a half months receiving an administration each day. As might also be expected, the liver suffered most of any organ when the chloroform was administered in this way. The amount of degenerative change was great in all cases, but was of a different type in those animals which lived for some time to that in those where death took place in a few days. In those dying soon the cells showed albuminous degeneration, vacuolation, and on two occasions all that could be made out of the liver cells was a kind of network outlining spaces which contained a poorly staining central nucleus surrounded by a small amount of coarsely granular protoplasm. In rabbit No.18, which lived for more than four months, there was a very great deal of fibrous tissue throughoutthe liver. Large openings were found irregularly distributed throughout the specimen, and the altered cells showed no sign of definite arrangement.

The greatest degenerative change was observed in No.

No.33, where the centres of the lobules had lost all trace of their cellular components and were made up of a cheesy mass containing numerous dark granules. Abundance of fat was always found.

The kidneys had on the whole suffered comparatively slightly, although in Nos.14 and 33 the cells in places were badly degenerated, showing but little sign of the original structure. In No,33 the glomerular tufts were shrunken and in a few places in the cortex as well as in the medulla the cells of the tubules were entirely lost. There was rarely congestion and no haemorrhages were found.

In the spleen the sinuses were found distended with blood, and pigment was present. In Nos.14, 15 and 23 the amount of iron shown by the ferrocyanide HCl stain was but small, although in 18 and 33 the amount was very great.

The large phagocytes were numerous in those specimens which contained much pigment.

Table VI. The Weights of the Spleens & other Particulars.

No.	Original weight of Animal	No.of days.	Weight of spleen.
14	2300 grammes	5	1.2 grammes
15	2150 "	14	0.3 gramme
18	2400 "	140	1.5 grammes
23	1700 "	30	0.95 gramme
33	2100 "	11	0.8 "

The average weight of the animal was 2130 grammes, while the average weight of the spleens was 0.95 gramme, the latter average being 0.17 gramme above the normal. The hearts showed very little variation from the normal, and the cross striping was seen plainly in all the specimens examined.

Consideration of Results.

In reviewing the results of the experiments detailed above, it appears to be proved that given in the form of small doses frequently repeated chloroform is a much more dangerous drug than when given in a single much larger dose. The first doses given appear to lower the vitality of the tissues, so that the later doses have a more marked action.

Idiosyncrasy or a number of undetermined factors appear to influence the action of chloroform on animals, and I found enormous variations between the shortest time and the longest time an animal lived during the administration.

In all the animals examined the liver appeared to have suffered greatly, although, as was to be expected, the most marked degeneration was found where the anaesthetic was

was given through the stomach. When the drug was inhaled the kidneys seem to have suffered more uniformly than in the other cases, and although the degree of degeneration was not always great, the difference in degree between kidney and liver degeneration was not nearly as marked as when the chloroform was given in either of the other ways.

Very great changes were seen almost uniformly in the spleen even in the animals that died in a few days. The evidence of extensive haemolysis was most marked. The large-sized phagocytes in many cases distended with dead red corpuscles and in other cases containing a pigment which gave a positive stain for iron, the masses of such pigment apparently lying loose in the spleen, and the large size and weight of the organ all demonstrated its increased activity (Paton and Goodall) (40). The organs in which this increased activity was least manifest were those removed from the rabbits which received the administration through the stomach much diluted with water. Here the average size of the spleen was small and the weights corresponded. The average weight was only 0.17 gramme above the normal, whereas in the other experiments the increase was 0.46 gramme when chloroform was inhaled and 0.59 gramme where it was injected.

When

When chloroform was inhaled it seemed to have a more marked action on the heart muscle than when it was given through the stomach or injected. Fat was not detected in the hearts examined.

In connection with the administration by the stomach, it is of interest to note that the amount of chloroform given in each administration was comparable with the quantity described by Sir James Crichton-Browne (3) as being present in one linseed, liquorice, and chlorodyne lozenge. The average weight of a full-grown rabbit is about 2 kilogrammes, while 70 kilogrammes represents the weight of a full-grown man. Thus 30 to 40 of these lozenges per diem in a man gives the same proportion of chloroform to body weight as in the experiments, and correspondingly less in a woman or a child. Many rabbits are very susceptible to the action of chloroform, but even in the one example where the animal lived for a long while the liver changes were of a very striking description. The lesions found in rabbits closely resemble those reported in fatal human cases of chloroform poisoning by Stiles and McDonald (41) and others.

The mortality among rabbits which have been anaesthetised, through the respiratory passages and the changes

changes in heart, liver, and kidney found after death emphasise the danger of repeated administration by this method, even when the anaesthetic is only exhibited for very short periods.

THE DANGERS OF CHLOROFORM ANAESTHESIA.

THE DANGERS OF CHLOROFORM ANAESTHESIA.

I. Immediate. The dangers of chloroform anaesthesia are not confined to those after-effects due to the alterations in histological structure, metabolism &c., detailed above, and which are referred to at length on page [illegible]. The prolonged administration of high percentages of chloroform undoubtedly cause death. Deaths also occasionally happen during the administration of the drug for short periods where the drug is not given in large quantities. When examining the cause of these deaths, certain physiological facts require to be considered.

Normal rhythmic breathing depends almost entirely upon the stimulation of the respiratory centre by the CO_2 in the blood; an increase of the CO_2 content, as, for example, during exercise, leads to hyperpnoea, whereas a diminution of the CO_2 causes apnoea. To this form of apnoea the name "acapnia" has been applied.

Although normally the degree of sensitiveness of the respiratory centre varies but little towards CO_2, Yandell Henderson

Henderson (42) has demonstrated that, under different forms and degrees of anaesthesia, variations from 50 per cent below to 50 per cent above the normal level may occur. He found that during light anaesthesia, and more especially where the administration was intermittent, the sensitiveness of the centre might be markedly increased, so that a lower percentage of CO_2 than normal caused hyperpnoea. In full anaesthesia, on the other hand, the centre reacted almost nornally, but its sensitiveness was much below the normal in very deep anaesthesia.

Such being the case, it is not difficult to appreciate the fact that where the anaesthetic has been given in an irregular and intermittent fashion, there is considerable danger to the patient, as, during the period of light anaesthesia, any struggling or deep breathing washes the CO_2 out of the blood, and when the anaesthesia becomes deep, with its accompanying lessening of the sensitiveness of the centre, a condition of acapnia can readily arise, which lasts until the accumulation of the CO_2 in the blood has reached the value necessary for the stimulation of the centre. This acapnic period may be quite short, or it may be so prolonged that the death of the patient results from deficient oxygenation - asphyxia. It may

may be remarked here that experimentally it has been shown, in the case of animals at least, that the use of artificial respiration by means of a pump, supplying air mixed with an appreciable amount of CO_2 is generally successful in resuscitating them from this acapnic condition. Henderson has pointed out further that anaesthesia started with ether and sustained with chloroform is likely to lead to untoward results, as the ether excitement readily produces hyperpnoea, with subsequent acapnia. He lays particular stress on the fact that fatalities rarely occur when the anaesthesia has been long and deep.

Levy (*43*) also published a number of papers relating to this question. He demonstrated that sudden death during chloroform anaesthesia was frequently due to ventricular fibrillation. Although, as a rule, death occurred during the induction of anaesthesia, cardiac irregularities could develop when alterations in the degree of anaesthesia took place - irregularities which were exactly similar to those preceding fibrillation. He further showed that ventricular fibrillation could be induced during light anaesthesia by the injection of small amounts of adrenalin into the blood-stream. He came to the conclusion that during

during light chloroform anaesthesia the heart becomes hypersensitive, and that any increase in the amount of adrenalin acts as an excitant to the production of ventricular irregularities. Deep anaesthesia, on the other hand, lowers the degree of cardiac sensitiveness. Levy noted further that the tendency to ventricular fibrillation was markedly increased when there had been any sensory excitation, or if the animal had struggled during the administration. His general conclusions are sufficiently noteworthy to quote in full.

1. The mammalian heart, when under the influence of chloroform, is in an "irritable" (shows a tendency to exhibit beats of a heterogenic origin) condition. This irritability is raised under conditions of light anaesthesia, and lowered under conditions of deep anaesthesia.

2. Abnormal ventricular beats are evoked in a heart under chloroform by conditions which stimulate it, or by equivalent conditions which remove or reduce depressing influences.

3. Under conditions of light chloroform anaesthesia, the ventricular irregularities resulting from cardiac stimulation may terminate in ventricular fibrillation and death of the heart.

4.

4. Stimulation of the heart may be effected - (a) as a reflex from sensory excitation; (b) as a result of an intermittent administration of the anaesthetic; (c) as a result of the state of nervous excitement, accompanied by struggling induced by chloroform in the earlier stages of its administration.

5. Ventricular fibrillation is a cause of death under chloroform, probably the only cause of any moment. It can be prevented by steadily maintaining a full degree of anaesthesia.

A practical point of considerable interest is that adrenalin may produce fibrillation of the heart if administered in any quantity during light anaesthesia.

Starling and von Anrep (44) had previously put forward the hypothesis that the rise in the blood-pressure which takes place in asphyxia was due to the fact that the increased CO_2 content of the blood stimulated the secretion of adrenalin. In this connection the further observations of Cannon and Hoskins (45) are of considerable interest and importance. These workers found that sensory excitation, nervous excitement, fear, all could stimulate the secretion of adrenalin. Finally, Levy showed that another factor

factor of great importance in the production of cardiac irregularities was the method of administration of the anaesthetic. If it were given in an irregular and intermittent manner there was grave danger, owing to sudden changes in the heart from a condition of hyper- to hyposensitiveness, and vice versa - a state of affairs which rendered the heart very subject to the induction of irregularities.

More recently Cathcart and the Author (46) have conducted a long series of experiments with the idea of determining the relative effects of CO_2, Lactic Acid, and other substances upon rabbits and dogs during light and full anaesthesia. In a large proportion of our experiments the anaesthetic used has been ether, but in some of them chloroform has been used. The effects are identical, but in the case of rabbits difficulty was experienced owing to the fact that the animals were particularly susceptible to the latter drug. This undue susceptibility was possibly an exaggeration of the normally increased "excitability" referred to by Levy (43) and showed itself in the form of fibrillation of the ventricle (1) when the concentration of the chloroform-air mixture was altered or (2) when the exhibition of the drug was momentarily stopped and then recommenced

recommenced. In the case of dogs, however, little difficulty was experienced.

Method.[1] In the experiments the animal under experiment was anaesthetised and a canula inserted into the trachea and connected with a Brodie respiration pump. A canula was tied into the left jugular vein, and in later experiments another into the right carotid artery which was connected with a Kymograph modified by McCall, our laboratory mechanic (47). The skin over the chest wall was removed and a cut into the thorax in the sixth intercostal space one inch to one and a half inches to the right of the border of the sternum. Artificial respiration was now commenced. The incision was now continued upwards parallel with the sternum to the second intercostal space. Next the fore-finger of the left hand was turned underneath the sternum, and the internal mammary vessels and sternum firmly held between the finger and thumb. The sternum was next cut across and the cut continued up the left side of the chest to the second left intercostal space. The sternum stillheld in the left thumb and forefinger was now turned

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[1] A demonstration of the method and of the influence of the carbon dioxide in varying depths of anaesthesia was given at the Internat.Congress of Med.1913, Pharmacol. Section

turned up and the internal mammary vessels ligated at a point as far up as was required. The sternum was now cut across and removed. The heart and pericardium lay exposed, and by carefully slitting up the pericardium and clipping its edges to the cut sides of the thorax, a cradle was prepared in which the heart lay without having its position seriously interfered with by the movements of the lungs. The advantage of this method over the more common method of exposing the heart by splitting the sternum lies first in its rapidity,and second in the fact that the operation can be carried out with practically no loss of blood.

The heart thus exposed was able to record its contractions on a smoked drum by levers attached by clips to the auricle and ventricle.

During the actual period of the experiments, artificial respiration was carried on by pumping with a hand bellows. The inlet of the hand bellows could be rapidly connected with a gas reservoir containing known dilutions of carbon dioxide and air (carbon dioxide and oxygen mixtures were sometimes employed with identical results). The carbon dioxide was obtained from a cylinder of compressed gas, and measured amounts of the mixture given. During the periods of hand pumping no further anaesthetic was

was given. In each case the hand pumping was started with ordinary air in order to get a comparative record,and when the trace was steady the carbon dioxide mixture was administered. In every instance the preliminary hand pumping with air was carried out by the same observer who gave the carbon dioxide mixture so that the results might be uniform as regards rate and force. It was found with a little practice to be very easy to keep up the hand pumping steadily at the same rate and pressure for the few minutes the experiment lasted. The earliest tracings were made with two ordinary levers, but the later ones were made by means of a very useful straight writing double recorder devised for use by the laboratory mechanic, J.McCall. In several of the later experiments the blood-pressure was recorded simultaneously with the cardiac contractions. During the intervals between experiments artificial respiration was carried out by means of the Brodie pump, the air passing to the animal through an anæsthetic bottle so arranged that the amount of the anaesthetic given could be regulated at will.

A.

TABLE I. *Light anæsthesia series.*

No. in series	Auricular & Ventricular *Rate*: Decrease 1st half	Decrease 2nd half	Increase 1st half	Increase 2nd half	Auricular and Ventricular *Amplitude*, in mm.: Decrease 1st half V	Decrease 1st half A	Decrease 2nd half V	Decrease 2nd half A	Increase 1st half V	Increase 1st half A	Increase 2nd half V	Increase 2nd half A	Total ventricular increase or decrease
1	2·5	0·0	—	—	5·0	1·0	10·0	—	—	—	—	0·8	−15·0
2	2·0	0·5	—	—	8·0	5·2	4·5	1·1	—	—	—	—	−12·5
3	0·0	3·0	—	—	2·6	—	3·4	7·0	—	0·6	—	—	− 6·0
4	3·25	2·75	—	—	19·0	6·4	—	10·0	—	—	0·6	—	−18·4
5	0·0	2·0	—	—	2·3	1·0	8·7	—	—	—	—	0·7	−11·0
6	2·0	2·0	—	—	9·0	—	—	—	—	—	1·7	—	− 7·3
7	1·5	2·0	—	—	2·3	1·1	15·4	1·6	—	—	—	—	−17·7
8	1·5	0·0	—	—	1·0	2·0	1·3	1·4	—	—	—	—	− 2·3
9	0·75	1·25	—	—	1·3	3·4	—	2·0	—	—	0·7	—	− 0·6
10	1·0	4·0	—	—	34·0	—	14·0	—	—	—	—	—	−48·0
	14·5	17·5	0·0	0·0	84·5	20·1	57·6	23·1	0·0	0·6	3·0	1·5	−138·8
Average for 10 Exps.:													
	1·45	1·75	0·0	0·0	8·45	2·01	5·76	2·31	0·0	0·06	0·3	0·15	−13·89

TABLE II. *Deep anæsthesia series.*

Degree of anæsthesia	No. in series	Auricular and Ventricular *Rate*: Decrease 1st half	Decrease 2nd half	Increase 1st half	Increase 2nd half	Auricular and Ventricular *Amplitude*, in mm.: Decrease 1st half V	Decrease 1st half A	Decrease 2nd half V	Decrease 2nd half A	Increase 1st half V	Increase 1st half A	Increase 2nd half V	Increase 2nd half A	Total ventricular increase or decrease
Deep ...	1	0·0	0·0	0·5	0·0	—	—	0·2	0·1	1·9	0·5	—	—	+1·7
Slight corneal reflex	2	1·5	0·0	0·0	0·0	4·1	0·6	2·0	—	—	—	—	2·0	−6·1
Deep ...	3	0·0	0·0	0·0	0·0	1·8	0·1	—	—	—	—	0·2	0·1	+1·6
Deep ...	4	0·5	0·0	0·0	0·0	1·0	—	—	—	—	—	—	—	−1·0
Deep ...	6	0·0	0·0	0·0	0·0	1·3	—	—	—	—	1·0	3·3	—	+2·0
Deep ...	7	0·0	0·0	0·0	0·0	0·2	—	—	—	—	—	1·4	2·2	+1·4
Slight C. R....	9	1·0	0·5	0·0	0·0	1·0	—	—	—	—	1·0	2·0	1·6	+1·0
Very deep ...	10	0·25	0·0	0·0	0·0	1·0	1·7	0·3	—	—	—	—	—	−1·3
Deep ...	11	0·5	0·5	0·0	0·0	--	0·4	—	—	—	—	—	0·4	0·0
C. R. after 20 secs.	12	0·25	1·25	0·0	0·0	0·3	—	0·3	—	—	0·6	—	1·0	−0·6
Slight C. R....	13	0·5	0·5	0·0	0·0	4·0	0·4	—	0·3	—	—	0·4	—	−3·6
Deep ...	14	0·0	0·0	0·0	0·0	8·0	2·0	—	—	—	—	1·0	1·0	−7·0
		4·6	2·75	0·5	0·0	22·7	5·2	2·8	0·4	1·9	3·12	8·3	8·3	−11·9
Av. for 12 Exps.		0·33	0·19	0·04	0·0	1·62	0·37	0·19	0·03	0·13	0·22	0·6	0·6	−0·85

Ether was used as an anaesthetic in this series. The results are identical with those obtained in the instances where chloroform was used. See, for example, Appendix IV.

A. Heart Experiments.

I. Influence of the anaesthetic alone. The influence of the anaesthetic alone is a point which had to be considered, and we carried out a number of observations on it. Our experiments have shown in common with the majority of others, carried out for this purpose, that, provided the administration is not pushed unduly, the anaesthetic has practically no effect on the rate and the amplitude of the cardiac beat. If it be given freely some inhibition of the amplitude takes place, and if it be given in excess the heart goes into a state of fibrillation, or a condition closely resembling it, from which it recovers more or less readily if artificial respiration be carried out with air free from the anaesthetic.

II. Influence of the inhalation of the carbon dioxide mixtures. The effect of the administration of varying mixtures of carbon dioxide and air to an anaesthetised animal varied according to the depth of the anaesthesia as is clearly demonstrated by the following protocols, tracings, and Tables I and II which give a summary of a series of the experiments. In addition to the experiments summarised

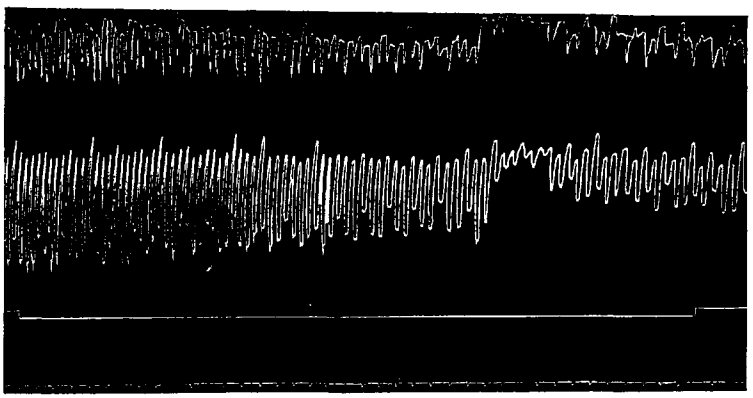


Fig. 1. Light anæsthesia. 3 litres of 16 % CO_2-air mixture.
[In this and all other traces the upper curves are auricular and the lower ventricular.]

summarised in these tables we have a very large number of experiments in which the results fall midway between deep and light anaesthesia as it is difficult to hit off always with certainty the proper depth of anaesthesia.

Protocol I. (a). The rabbit was lightly anaesthetised, and a mixture containing 16% carbon dioxide in air was given. The amplitude of the excursion of both ventricular and auricular levers diminished markedly within ten seconds of the commencement of the experiment and the rate became slowed (see Fig.1). After 30 seconds a slight struggle took place, and the condition of the heart was so unsatisfactory that the experiment was stopped after three litres of the carbon dioxide air mixture had been given. The heart had been gradually failing, and the marked dilatation of the auricles and the ventricles gave the trace a diastolic character. The measurements of the rate and amplitude are as follows:

Rate

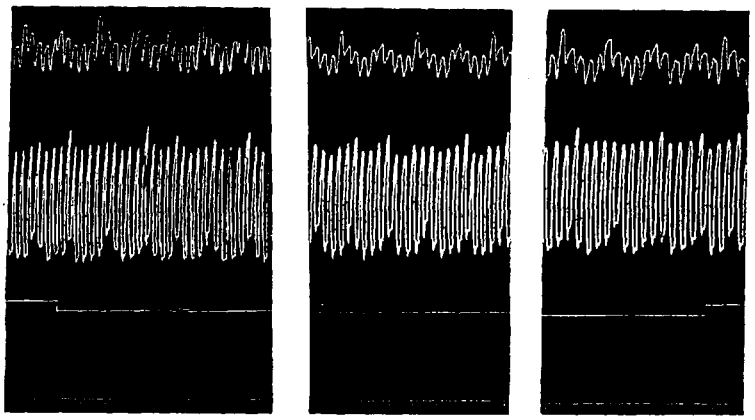


Fig. 2. Deep anæsthesia. 5 litres of 16 % CO_2-air mixture. Intervals = 20 secs. Total time = 72 secs.

Rate		Commencement of experiment	Middle of experiment	End of Experiment.
Ventricle)	) in 3 seconds	8.5	7.0	5.0
Auricle)		8.5	7.0	5.0
Amplitude.				
Ventricle)	average of 3 con-	29.0 mm.	26.7 mm.	11.3 mm
Auricle)	tractions	8.3	7.3	5.7
Height of lowest point of ventricular trace above the base line.		30.0 mm.	38.0 mm.	46.0 mm.

(For tracings from this and other experiments in this series see Appendix)

(b) At the conclusion of Exp. (a) the anaesthetic was given until the animal was deeply under, no corneal reflex, the pupil widely dilated and inactive. Again 16% carbon dioxide air mixture was given as before. A very slight diminution in rate took place, but no change in the amplitude. Five litres of the mixture were given without markedly influencing the heart (see Fig.2). The measurements of the rate and the amplitude are as follows:

Rate

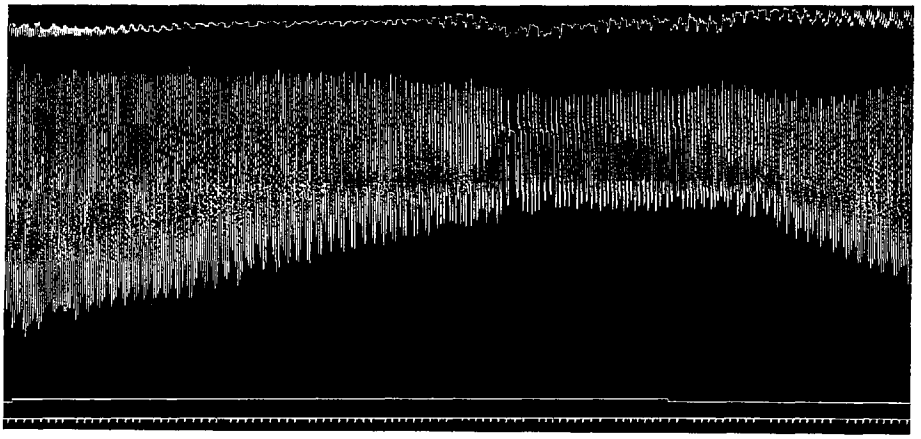


Fig. 3. Light anæsthesia. 5 litres of 12 % CO_2-air mixture.

Rate		Commencement of experiment	Middle of experiment	End of experiment.
Ventricle) in 3 seconds	...	9.0	8.5	8.0
Auricle)	...	9.0	8.5	8.0
Amplitude.				
Ventricle) Average of 3 con-		21.0 mm.	21.0 mm.	21.0 mm.
Auricle) tractions		4.7	4.3	4.7
Height of lowest point of ventricular trace above the base line.		22.0 mm.	23.0 mm.	24.0 mm.

Protocol II. (a) A rabbit was lightly anaesthetised and five litres of a mixture of 12% carbon dioxide in air were given. The amplitude of the auricular and ventricular contractions diminished slowly during the first 30 seconds after the administration began and then more rapidly, reaching a minimum 70 seconds from the commencement of the administration. The rate was slowed slightly at first but markedly during the second half of the administration (see Fig.3). There was marked dilatation of the heart. Measurements were as follows:

Rate

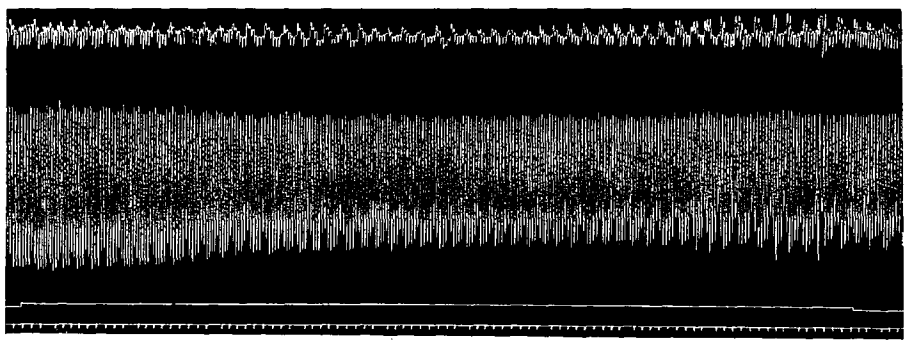


Fig. 4. Deep anæsthesia. 5 litres of 12 % CO_2-air mixture.

Rate		Commencement of experiment	Middle of experiment	End of experiment
Ventricle	in 3 seconds ...	11.0	10.0	6.0
Auricle	in 3 seconds	11.0	10.0	6.0
Amplitude				
Ventricle	average of 3 contractions	85.0 mm.	51.0 mm.	37.0 mm.
Auricle	average of 3 contractions	4.0	-	-
Height of lowest point of ventricular trace above the base line.		24.0 mm.	47.0 mm.	64.0 mm.

(b) At the close of the above experiment the administration was pushed until the animal was deeply anaesthetised. No corneal reflex, and pupils dilated and inactive. Five litres of 12% carbon dioxide air mixture were given. A slight diminution in amplitude of the heart's contraction took place at first to be followed by a very small increase but no change in the rate (see Fig.4). The measurements were:

Rate		Commencement of experiment	Middle of experiment	End of experiment
Ventricle	in 3 seconds ...	8.0	8.0	8.0
Auricle	in 3 seconds ...	8.0	8.0	8.0
Amplitude				
Ventricle	average of 3 contractions	52.0 mm.	44.0 mm.	45.0 mm.
Auricle	average of 3 contractions	6.0	4.0	5.0
Height of lowest point of ventricular trace above the base line.		55.0 mm.	62.0 mm.	63.0 mm.

The results which we have obtained may be briefly summarised as follows. When the animal is lightly under the influence of an anaesthetic (in all cases the animals were quite unconscious) the effect of the administration of carbon dioxide, by way of the respiratory tract, produces, with almost perfect regularity, a reduction both in the rate and the amplitude of the heart beat, whereas when the animal is deeply under (when the administration of the anaesthetic was pushed until the cardiac contraction was slightly affected) there is no, or at most merely the slightest, reduction in the rate and amplitude of the heart beat. That this inhibitory influence of the depth of the anaesthesia is a sensitive balance is shown by the fact that when a series of experiments are carried out in succession, beginning with the animal deeply anaesthetised and continuing without further administration there is a steady increase in the carbon dioxide poisoning effect as demonstrated by the gradual decrease in the amplitude of the heart contraction, particularly of the ventricle.

In view of the results obtained by Starling and his co-workers it was of interest to investigate the effect of the administration of carbon dioxide in varying depths of

of anaesthesia on the blood-pressure in addition to the heart direct.

Before determining the influence of the addition of the carbon dioxide we carried out a series of experiments on the direct influence of the anaesthetic alone on the blood pressure. We found in a number of our experiments that on changing from "light" to "very deep" anaesthesia the blood-pressure might fall as much as 50 mm. of Hg. and that a corresponding rise in blood-pressure took place when the anaesthesia changed from "deep" to "light".

The effect of giving carbon dioxide was very marked in that, as the anaesthesia became less deep, the administration of the carbon dioxide air mixture brought about a gradual increase in the blood-pressure. In stages of light anaesthesia the carbon dioxide frequently caused a sharp rise of from 30 to 40 mm.Hg. from the base line at the commencement of the administration. Further, that the blood-pressure rise continued after the administration of the carbon dioxide had ceased, in other words that the apex of the blood-pressure rise was not synchronous with the point of minimum amplitude of the auriculo-ventricular trace. In deep anaesthesia this rise in blood-pressure was not

not brought about by the giving of carbon dioxide. On the other hand when the anaesthesia was less deep but still deep enough to prevent the carbon dioxide affecting very definitely the rate and the amplitude of the heart a rise in blood pressure might occur.

As regards the relationship of the rise and fall in blood-pressure to the variation in the amplitude and rate of the cardiac contraction our experiments tend to show that, although there may be some connection, the diminished amplitude which results from the administration of carbon dioxide during light anaesthesia is not solely dependent upon the alteration of the blood-pressure. The following short summary and the subsequent protocols will serve to make these points clear.

Light anaesthesia + CO_2. Marked rise in blood-pressure. Labouring heart. Blood-pressure starts to rise before the heart trace becomes affected and the apex of the B.P.curve is frequently subsequent to the point of minimum amplitude of the cardiac contraction: it may even occur after the cessation of the administration of the CO_2.

Medium anaesthesia + CO_2. Rise in blood-pressure. No marked labouring of the heart although slight diminution in

in the amplitude may take place.

Deep anaesthesia + CO_2. No rise in blood-pressure. Practically no alteration of amplitude of the cardiac contraction.

The following experiments may also be cited:

Protocol III. The animal was very deeply anaesthetised and a series of tracings without further anaesthesia were taken at intervals of about four minutes. (The experimentwas stopped before the animal came out of the anaesthetic).

(a) Animal deeply under. Five litres of 12% carbon dioxide air mixture given. The auricular contraction was poor before the carbon dioxide was administered but commenced to improve after about one minute and was quite good at the end of the administration. The ventricular trace showed a slight diminution in amplitude for some 25 seconds, but subsequently increased to its initial size. The blood-pressure rose very slightly at first but not again until more than half of the carbon dioxide had been given when a second slight rise took place. In all the rise in blood-pressure from the beginning of the experiment until it

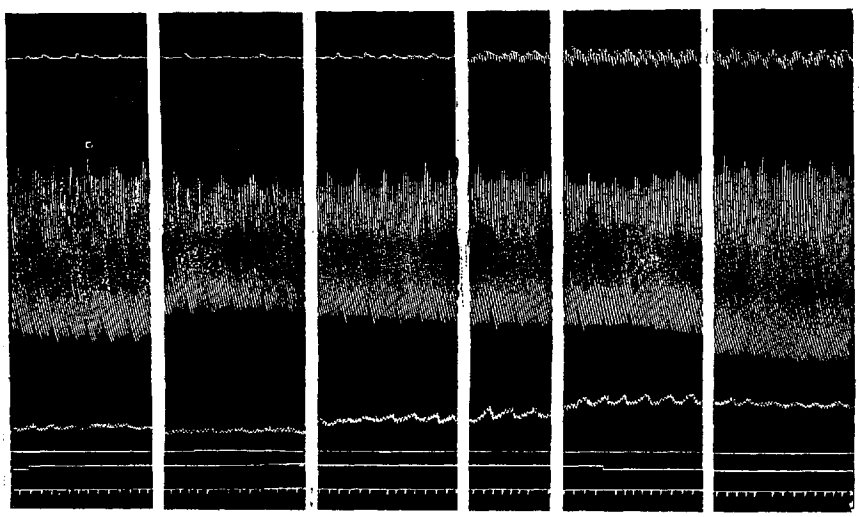


Fig. 5. Deep anæsthesia. Heart contraction and blood-pressure recorded simultaneously. 5 litres of 12 % CO_2-air mixture. Intervals = 14 secs. Total time = 111 secs.

it reached its maximum height about 20 seconds after the cessation of the administration of the carbon dioxide was only about 12 mm.of Hg. (see Fig.5). After the carbon dioxide was all given and pure air was administered the cardiac amplitude rapidly increased, but the blood-pressure after reaching its maximum very gradually fell away. The figures were:

Rate	Commencement of experiment	Middle of experiment	End of experiment
Ventricle } in 3 seconds ...	13.0	13.0	13.0
Auricle }	?	13.0	13.0
Amplitude.			
Ventricle } average of 3 contractions	46.0 mm.	40.3 mm.	43.1 mm.
Auricle }	?	1.0	3.0
Height of lowest point of ventricular contraction above the base line.	76.0 mm.	84.0 mm.	80.0 mm.
Blood-pressure in mm.Hg.above base line.	25.0 mm.	27.0 mm.	35.0 mm+

+ 30 seconds later 37.0 mm.

(b) No.5 in series. Animal lightly anaesthetised and the heart beating well. When five litres of 12% carbon dioxide air mixture were given both the ventricular and the auricular contractions diminished in amplitude, the rate

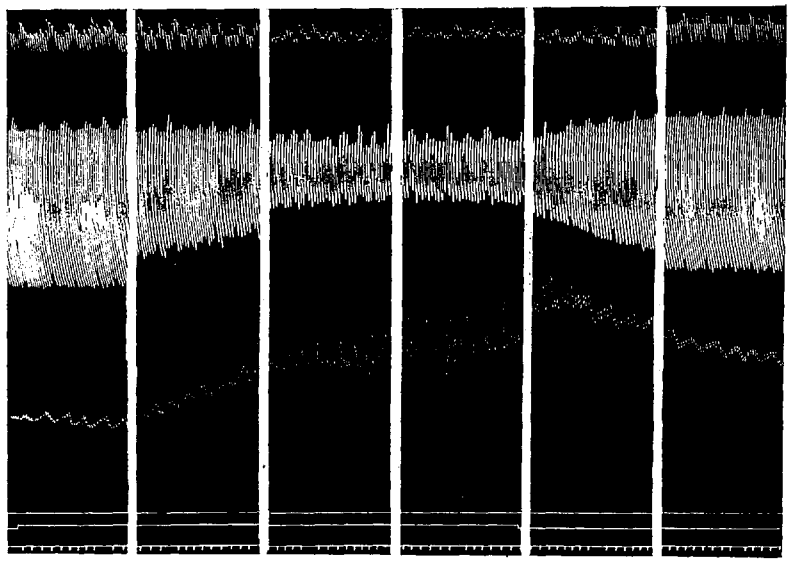


Fig. 6. Light anæsthesia. Heart contraction and blood-pressure recorded simultaneously. 5 litres of 12 % CO_2-air mixture. Intervals = 14 secs. Total time = 100 secs.

rate slowed but the blood-pressure rose some 30 seconds after the commencement of the experiment. After the administration of the carbon dioxide had ceased, the heart began to contract better and the rate increased again, the blood-pressure, however, continued to rise for a further 30 seconds and reached its maximum at a point when the ventricular and auricular traces were rapidly increasing in amplitude (see Fig.6.). The figures were:

	Commencement of experiment	Middle of experiment	End of experiment
Rate			
Ventricle) in 3 seconds	13.0	8.0	8.0
Auricle)	13.0	8.0	8.0
Amplitude			
Ventricle) average of 3 con-	54.3 mm.	21.7 mm.	21.3 mm.
Auricle) tractions	7.7	1.8	1.3
Height of lowest point of ventricular trace above the base line.	78.0 mm.	101.0 mm.	101.0 mm.
Blood-pressure in mm.Hg.above base line.	52.0 mm.	68.0 mm.	77.0 mm+

+ 30 seconds later, 90 mm.

Thus it would appear that when the anaesthesia is deep, so deep that carbon dioxide has practically no effect on the amplitude of the heart beat, there is no marked rise in

in the blood-pressure in spite of the fact that the percentage of carbon dioxide used ought, if the explanation of Starling and von Anrep be the correct one, to have evoked a secretion of adrenalin with its accompanying rise in blood-pressure; and yet, with the light anaesthesia, when the amplitude is affected, we find the rise in blood-pressure noted by Starling.

At present we are carrying out a further series of experiments with a view to elucidating the manner in which the anaesthesia exercises its inhibiting action. We find that, however deep the degree of anaesthesia, injections of minute quantities of adrenalin still yield a normal rise of blood pressure, even after the administration of large doses of Atropin, Nicotin, and Curara. That the absence of rise of pressure on administration of CO_2 during deep anaesthesia is due to the paralysis of a central mechanism is supported by the fact that decapitation of an animal is followed by a fall of blood pressure which does not rise in a normal fashion on administration of CO_2, even an hour and more after the anaesthetic was last administered. Here also, the injection of a minute quantity of an extract of supra-renal medulla is followed by a normal rise in blood pressure.

As

As regards the practical aspect of our work, it certainly gives support to the view that if anaesthesia is to be carried out safely it must be deep.

The results of our experiments with lactic acid are inconclusive, and we are continuing this series.

2. Remote. The facts considered above show clearly that the administration of chloroform is followed by changes in the tissues and that consequent upon this there are alterations in metabolism and loss of weight. These effects are generally short lived and even after relatively great changes in the liver, complete recovery may take place in rabbits and dogs. In some instances, however, this is not the case, and the animal dies days or sometimes weeks after the administration. This condition is termed "delayed chloroform poisoning". In searching for the particular element involved, the author was struck by the fact that in the human subject changes in the liver were found after death from delayed chloroform poisoning which were identical with those found in animals where the drug was injected subcutaneously or given by mouth. An investigation was consequently undertaken to discover if there was any difference in the distribution of the chloroform in the blood when given in these ways from that found after inhalation. The results are referred to on page 14 et seq. It is thus probable that the delayed elimination of the drug is due to the fact that a larger amount is combined in the plasma. This solution of chloroform in plasma bathes the tissues

tissues and exerts its effect upon the cells for an unduly long time. The chloroform combined with the lipoids of the red corpuscles is rapidly assumed and rapidly eliminated, that in the plasma - whether in solution or combined with proteins or lipoids is uncertain - is slowly eliminated. Chloroform evidently combines loosely with the corpuscular element and firmly with the plasma element. The anaesthetic effect is obtained by the loosely-combined portion, the destructive effect largely by the more firmly combined portion whose elimination is delayed. It is instructive to note that in an animal, which was injected with 1 c.c. of chloroform and killed after five and a half hours, 31.6 mg. of chloroform per cent were found in the blood; while the animal, though shaky, was not completely anaesthetised, whereas full anaesthesia was produced by inhalation in a rabbit whose blood contained 30.8 mg. per cent when killed immediately after the administration. Another rabbit, which had 1 c.c. administered by the stomach, and which was also not fully anaesthetised, showed 32.5 mg. per cent in the blood when killed three hours after.

The total quantity of chloroform in the blood is not therefore a measure of the degree of anaesthesia. Complete

Complete anaesthesia is obtained with a much smaller amount of chloroform in the blood when the anaesthetic is given by inhalation than when given in the two other ways mentioned.

Thus during its administration the dangers of chloroform as an anaesthetic are such as may be surmounted by care in rate of administration. Due recognition of the importance of rendering the anaesthesia an efficient one must be recognised. After its administration, any treatment which will increase the rate of elimination is to be recommended. The habit of letting a patient "sleep off" the drug is not likely to expedite elimination, but rather the reverse, and this should therefore not be permitted.

The Appendices following contain some of the figures, photographs and records obtained in the experiments quoted above. No attempt has been made to make these exhaustive.

APPENDIX I.

Influence of Chloroform on the Tissues.

I. By Inhalation.

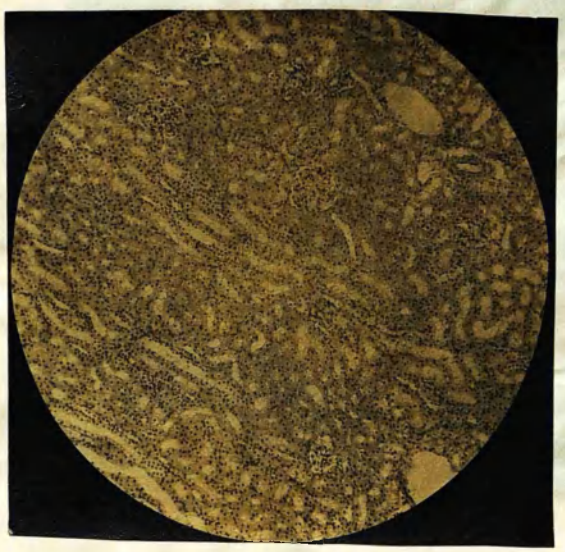


Fig.1. Kidney from rabbit No.28 of the series. Chloroform was administered through the respiratory passages for fifteen minutes and the animal was killed one hour after. There is but little variation from the normal. X 66.

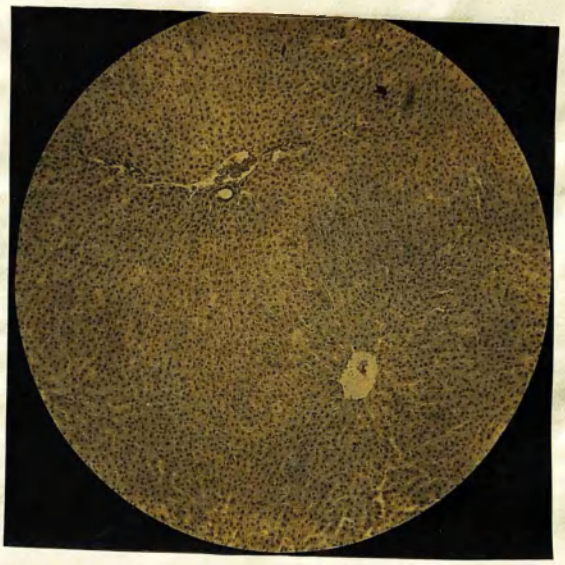
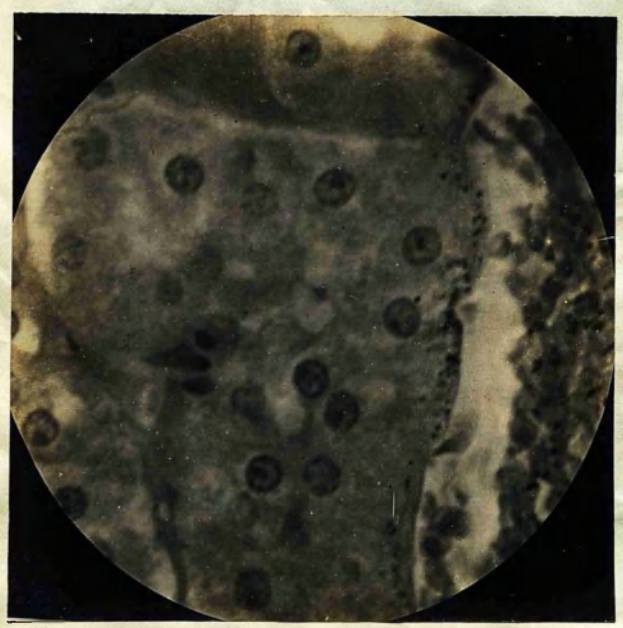


Fig.II. Liver from the same animal as Figure I. The organ shows a normal appearance. X 66.

2. By Stomach.

Fig.iii. Kidney from rabbit No.9a of the series which had 1 c.c. of chloroform administered in oil by the stomach. To the right is seen a quantity of extravascular blood. The cells lining the tubules are in various states of degeneration. The nuclei as a whole are badly stained. X 150.



Fig,iv. High power view of the tubules adjacent to the extravascular blood. The granules referred to in the text are well shown. X 800.

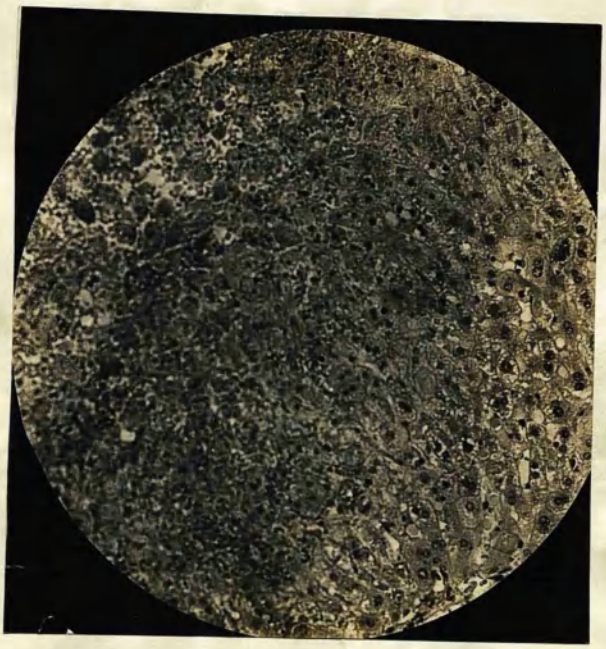


Fig.v. Liver from the same rabbit. To the left the centre of the lobule is seen to be completely broken down while to the right the cells at the periphery appear almost normal. X 150.

3. By Injection.

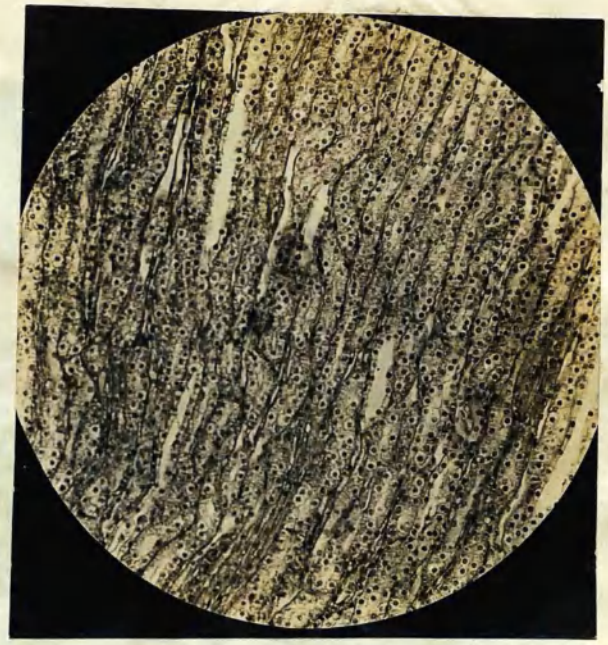


Fig. vi. Medulla of kidney from rabbit No.33 which received an injection of 1 c.c. of chloroform subcutaneously. A considerable degree of degeneration is observable and the nuclei are losing the power of taking on the basic stain. X 150.

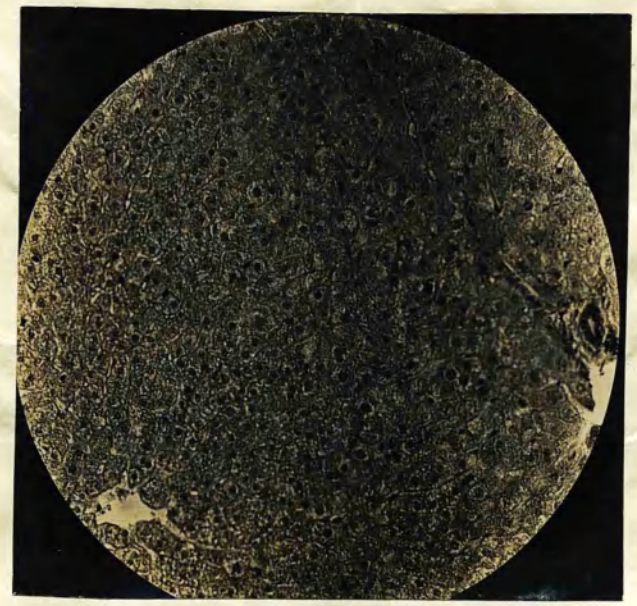


Fig.vii. Liver from the same animal. The centre of the lobule shows cells considerably degenerated and even vacuolated. The periphery - to the right - shows cells which are almost normal in appearance. X 150.

APPENDIX II.

Influence on Red Blood Counts.

Numbers given per cubic millimeter.

1. Inhalation.

1. Inhalation

Rabbit II. 1300 grms.chloroform given for 15 minutes

Before chloroform	5,200,000
1st.day after	5,600,000
2nd. " "	6,000,000
3rd. " "	5,500,000
4th. " "	5,600,000
5th. " "	5,200,000

Rabbit XVI. 1330 grms.chloroform given for 30 minutes

Before chloroform	5,360,000
1st.day after	6,240,000
2nd.day after	6,500,000
3rd. " "	6,430,000
4th. " "	5,964,000
5th. " "	5,580,000

Rabbit XIX. Wt. 1750 grms.chloroform for ½ hour.

Before chloroform	6,900,000
1st.day after	8,240,000
2nd. " "	8,400,000
3rd. " "	7,480,000
4th. " "	7,360,000
5th. " "	7,600,000
6th. " "	6,960,000

Rabbit XX. Wt. 1100 grms.chloroform for ½ hr.

Before chloroform	5,840,000
1st.day after	6,560,000
2nd. " "	7,200,000
3rd. " "	6,400,000
4th. " "	5,600,000

Rabbit XXII. Wt. 1000 grms. chloroform for ½ hour.

Before chloroform	6,080,000
1st. day after	7,440,000
2nd. " "	7,560,000
3rd. " "	6,600,000
4th. " "	5,600,000

Rabbit XXVI. Wt. 2,000 grms. Chloroform for ½ hour.

Before chloroform	6,000,000
1st. day after	7,280,000
2nd. " "	7,240,000
3rd. " "	7,620,000
4th. " "	6,320,000
5th. " "	6,440,000
6th. " "	6,210,000

RabbitXXIX Wt. 960 grm. Chloroform for ½ hour.

Before chloroform	5,680,000
1st. day after	5,760,000
2nd. " "	4,080,000 died.

Rabbit XXXII. Wt. 1250 grms. Chloroform for 1 hour.

Before chloroform	5,440,000
1st. day after	4,250,000
2nd. " "	4,720,000
3rd. " "	4,450,000
4th. " "	4,830,000
5th. " "	4,300,000
6th. " "	4,400,000
7th. " "	died.

Rabbit XXXV. Wt. 1465 grms. Chloroform for ½ hour

Before chloroform	5,320,000
1st.day after	6,560,000
2nd. " "	6,340,000
3rd. " "	5,640,000
4th. " "	5,470,000

Rabbit XXXVII. Wt. 2000 grms. Chloroform for 1 hour.

Before chloroform	6,230,000
1st.day after	7,030,000
2nd. " "	6,460,000
3rd. " "	6,660,000
4th. " "	6,060,000
5th. " "	6,580,000

Rabbit XXXVIII. Wt. 2,000 grms. Chloroform for 1 hour

Before chloroform	7,090,000
1st.day after	7,730,000
2nd.day after	7,670,000
3rd. " "	7,120,000
4th. " "	6,360,000
5th. " "	6,900,000

Rabbit XXXIX. Wt. 1750 grms. Chloroform for 1 hour.

Before chloroform	7,360,000
1st.day after	6,420,000
2nd.day after	4,870,000
3rd. " "	4,310,000
4th. " "	died

Rabbit XL. Wt. 1900 grms. Chloroform for 1 hour

Before chloroform	5,760,000
1st.day after	
2nd. " "	6,490,000
3rd. " "	6,590,000
4th. " "	6,080,000

Rabbit XLI. Wt. 1560 grms. Chloroform for 1 hour.

Before chloroform	6,800,000
1st.day after.	7,744,000
2nd. " "	6,760,000
3rd. " "	6,240,000
4th. " "	5,920,000
5th. " "	5,920,000

Rabbit XLIII. Wt. 1256 grms. Chloroform for ½ hour.

Before chloroform	6,750,000
1st.day after	7,440,000
2nd. " "	6,940,000

2. Injection.

2. Injection.

Rabbit V. Wt. 1850 grms. 1 c.c. chloroform injected

Before chloroform	6,320,000
1st.day after	7,500,000
2nd. " "	6,600,000
3rd. " "	6,400,000
4th. " "	6,800,000
5th. " "	6,300,000

Rabbit VI. Wt. 2100 grms. 1 c.c. chloroform injected

Before chloroform	5,600,000
1st.day after	4,800,000
died.	

Rabbit VIII. Wt. 1240 grms. 1 c.c. Chloroform injected

Before chloroform	5,600,000
1st.day after	7,840,000
2nd. " "	7,600,000
3rd. " "	5,680,000
4th. " "	6,400,000
5th. " "	6,600,000
6th. " "	6,320,000

Rabbit IX. Wt. 1300 grms. 1 c.c. Chloroform injected

Before chloroform	5,600,000
1st.day after	6,700,000
2nd.day after	5,850,000
3rd. " "	6,160,000
4th. " "	5,840,000
5th. " "	5,400,000

Rabbit X. Wt. 1300 grms. 1 c.c. Chloroform injected.

Before chloroform	5,600,000
1st.day after	5,600,000
2nd. " "	died

Rabbit XII. Wt. 2360 grms. 1 c.c. Chloroform injected

Before chloroform	5,760,000
1st.day after	6,500,000
2nd. " "	6,720,000
3rd. " "	5,840,000
4th. " "	6,000,000
5th. " "	5,600,000
6th. " "	5,920,000

Rabbit XIII. Wt. 1850 grms. 1 c.c. Chloroform injected.

Before Chloroform	5,040,000
1st.day after	5,120,000
2nd. " "	5,480,000
3rd. " "	died

Rabbit XIV. Wt. 1900 grms. 1 c.c. Chloroform injected

Before chloroform	5,400,000
1st. day after	6,960,000
2nd. " "	6,640,000
3rd. " "	6,080,000
4th. " "	6,400,000

Rabbit XV. Wt. 1760 grms. 1 c.c. Chloroform injected

Before chloroform	5,400,000
1st.day after	6,480,000
2nd.day after	6,640,000
3rd. " "	6,500,000

Rabbit XVIII. Wt. 2300 grms. 1 c.c. Chloroform injected

Before chloroform	6,400,000
1st.day after	7,440,000
2nd.day "	7,520,000
3rd. " "	6,480,000

Rabbit XXI. Wt. 1050 grms. 1 c.c. Chloroform injected

Before chloroform	5,600,000
1st.day after	7,440,000
2nd. " "	7,200,000
3rd. " "	7,080,000

Rabbit XXIV. Wt. 2300 grms. 1 c.c. Chloroform injected

Before chloroform	6,320,000
1st.day after	8,880,000

Rabbit XXV. Wt. 2000 grms. 1 c.c. Chloroform injected

Before chloroform	6,080,000
1st.day after	7,840,000

Rabbit XXVII. Wt. 1400 grms. 1 c.c. chloroform injected

Before Chloroform	6,800,000
1st.day after	7,490,000
2nd. " "	6,900,000

Rabbit XLII. Wt. 1550 grms. 1 c.c. Chloroform injected

Before Chloroform	6,790,000
1st.day after	8,200,000
2nd. " "	7,230,000
3rd. " "	7,440,000
4th. " "	7,520,000

3. By Stomach.

3. By Stomach.

Rabbit XXVIII. Wt. 1560 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before chloroform	6,400,000
1st.day after	5,280,000
2nd. " "	5,960,000
3rd. " "	4,040,000
4th. " "	4,160,000
5th. " "	4,680,000

Rabbit XXIX$_A$ Wt. 1800 grms. 1 c.c. Chloroform in 10 c.c Olive Oil.

Before Chloroform	6,320,000
1st.day after	7,040,000
2nd. " "	6,320,000
3rd. " "	6,720,000
4th. " "	5,600,000
5th. " "	5,980,000
6th. " "	6,040,000

Rabbit XXXI. Wt. 1140 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before Chloroform	5,120,000
1st.day after	5,360,000

Rabbit XXXIII. Wt. 1650 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before Chloroform	5,460,000
1st.day after	7,300,000
2nd.day "	5,480,000
3rd. " "	6,160,000
4th. " "	6,020,000
5th. " "	6,070,000
6th. " "	6,560,000

Rabbit XXXVI. Wt. 1150 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before Chloroform	5,840,000
1st.day after	6,720,000
2nd. " "	6,480,000
3rd. " "	5,880,000
4th. " "	5,770,000

Rabbit XLIV. Wt. 1750 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before chloroform	7,600,000
1st.day after	8,800,000
2nd. " "	7,600,000

Rabbit XLV. Wt. 1725 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before chloroform	6,400,000
1st.day after	7,940,000
2nd. " "	7,280,000
3rd. " "	7,460,000
4th. " "	7,200,000
5th. " "	6,400,000
6th. " "	6,240,000
7th. " "	6,400,000

Rabbit XLVI. Wt. 1900 grms. 1 c.c. Chloroform in 10 c.c. Olive Oil

Before chloroform	6,160,000
1st.day after	7,820,000
2nd. " "	8,240,000
died	

Rabbit LI. Wt. 1350 grms.

Daily counts were made for 8 days as a control with an average variation of 250,000, 1 c.c. chloroform in 10 c.c. Olive Oil was now given.

Before chloroform	7,360,000
1st.day after	7,248,000
died	

Rabbit LIII. Wt. 1800 grms.

Daily counts were made for 6 days as a control before 1 c.c. of chloroform in 10 c.c. of Olive Oil was given. Average variation 180,000.

Before chloroform	5,200,000
1st.day after	4,800,000
2nd. " "	4,528,000
died.	

Rabbit LVI. Wt. 1850 grms.

Daily counts were made for 4 days before 1 c.c. of chloroform in 10 c.c. of Olive Oil was given. Average variation, 240,000.

Before chloroform	5,840,000
1st.day after	7,600,000
2nd. " "	8,450,000
3rd. " "	8,240,000
4th. " "	7,670,000

Rabbit LVII. Wt. 1900 grms. 1 c.c. chloroform in 10 c.c. Olive Oil.

Before chloroform	6,060,000
1st.day after	6,400,000
2nd. " "	6,100,000
3rd. " "	6,400,000
4th. " "	6,320,000

APPENDIX III.

Specific Gravity of Blood Serum from Normal Rabbits and from Rabbits which were injected with 1 c.c. of Chloroform subcutaneously.

The chloroform rabbits were killed 24 hours after the injection was given.

In these experiments the animals were taken directly from their cages and, having been killed by a sharp blow behind the occiput, were rapidly bled from the jugular and carotid vessels into an open dish. The blood remained in this dish in a moist atmosphere for varying times as detailed below. The supernatant serum was withdrawn and its Specific Gravity estimated by means of a Specific Gravity Bottle. In some instances the total protein content of the serum in the bottle was ascertained. As the experiments were devised to determine if the polycythaemia observed to follow an administration of chloroform was due to a concentration of the blood by increased excretion of urine, the information would be given in the affirmative if the Specific Gravity were constantly higher in chloroformed animals than normal. The protein content of the serum would also be constantly higher in chloroformed animals if this theory is correct.

1. Normal.

A. Normal rabbit. Wt. 1150 grms.

Bled into open dish, serum removed and Specific Gravity estimated 24 hours later.

Specific Gravity - 1024.

B. Normal rabbit. Wt. 1300 grms.

Bled as above. Blood lay in dish for 24 hours.

Specific Gravity - 1026.5

Protein content of 10 ccs.Serum - 0.644 grm.

C. Normal Rabbit. Wt. 1200 grms.

Bled as above. Specific Gravity of Serum taken after 24 hours.

Specific Gravity - 1024.

Protein Content of 10 ccs.Serum - 0.679 grms

G. Normal Rabbit. Wt. 960 grms.

Bled as above, lay in dish 6 hours.

Specific Gravity - 1018.6

Protein Content of 10 ccs.serum - 0.559 grms.

H. Normal Rabbit. Wt. 1480 grms.

Blood lay in dish 6 hours.

Specific Gravity - 1022.3

Protein Content of 10 ccs.Serum - 0.544 grms.

K. Normal Rabbit. Wt. 1150 grms.

Blood lay in dish 12 hours.

Specific Gravity - 1017.5

Protein content of 10 ccs.serum - 0.517 grms.

O. Normal rabbit. Wt. 1350 grms.

Blood lay in dish 6 hours.

Specific Gravity - 1016.6

Protein content of 10 ccs. serum - 0.517 grms.

U. Normal rabbit. Wt. 1320.

Blood lay in dish 24 hours.

Specific Gravoty 1022.4

Protein content of 10 ccs.serum - 0.467. grms.

The average Specific Gravity in the 8 animals referred to above is 1021.5, and the average protein content in 10 ccs. of serum in the 7 specimens estimated is 0.561 grms.

2. After Chloroform.

D. Rabbit. Wt. 1100 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 24 hours.

Specific Gravity - 1026.

Protein content of 10 ccs. Serum - 0.815 grms.

F. Rabbit. Wt. 1450 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 6 hours.

Specific Gravity - 1028

Protein content of 10 ccs. Serum - 0.708 grms.

I. Rabbit. Wt. 1200 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 6 hours.

Specific Gravity - 1029.8.

J. Rabbit Wt. 1250 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 24 hours.

Specific Gravity - 1028.

Protein content of 10 ccs. Serum - 0.749 grms.

M. Rabbit Wt. 1650 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish for 12 hours.

Specific gravity - 1026.4

A.A. Rabbit. Wt. 2000 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 12 hours.

Specific gravity - 1024.

Protein content of 10 ccs. serum - 0.657 grm.

A.B. Rabbit. Wt. 2500 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 12 hours.

Specific Gravity - 1026.4

Protein content of 10 ccs. serum - 0.628 grm..

A.D. Rabbit. Wt. 2300 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 12 hours.

Specific gravity - 1024.3.

Protein content of 10 ccs. serum - 0.68 grm..

A.H. Rabbit. Wt. 1450 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 24 hours.

Specific Gravity - 1025

Protein content of 10 ccs. serum - 0.679 grm.

A.J.

A.J. Rabbit. Wt. 1900 grms.

1 cc. injected into flank, animal killed 24 hours later.

Blood lay in dish 24 hours.

Specific Gravity - 1027.2.

Protein content of 10 ccs. serum - 0.768 grm.

The average Specific Gravity in the 10 animals referred to above is 1026.5 and the average protein content in 10 ccs. of serum in the 8 specimens estimated is 0.712 grm.

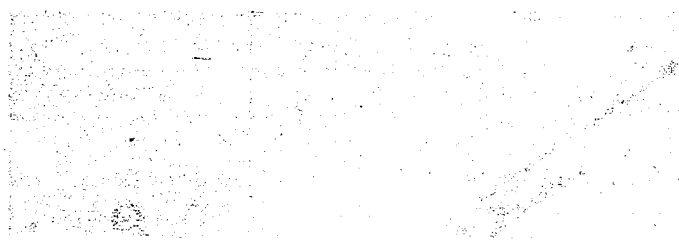
APPENDIX IV.

Details of an experiment on a dog showing the influence of depth of Chloroform Anaesthesia upon the effects of Carbon Dioxide.

The animal was deeply anaesthetised with chloroform and its trachea exposed, opened, and a canula inserted. The canula was connected through a chloroform bottle with a Brodie respiration pump. The carotid artery was connected by a canula with a Kymograph and a canula tied into the jugular vein. Hirudin was injected to prevent clotting. The subsequent procedure was exactly as detailed on page 69 et seq.

The protocols illustrate clearly the influence of depth of anaesthesia upon (a) blood pressure, and (b) amplitude of movement of the lever attached to the ventricle.

The records are continuous.



Protocol I. Lightly under.

Blood Pressure - 60 mm. Hg.

Ventricular amplitude - 30 m.m.

4 Litres 12% CO_2 - Air Mixture given.

At end of administration lasting 90 seconds.

Blood Pressure - 80 m.m. Hg.

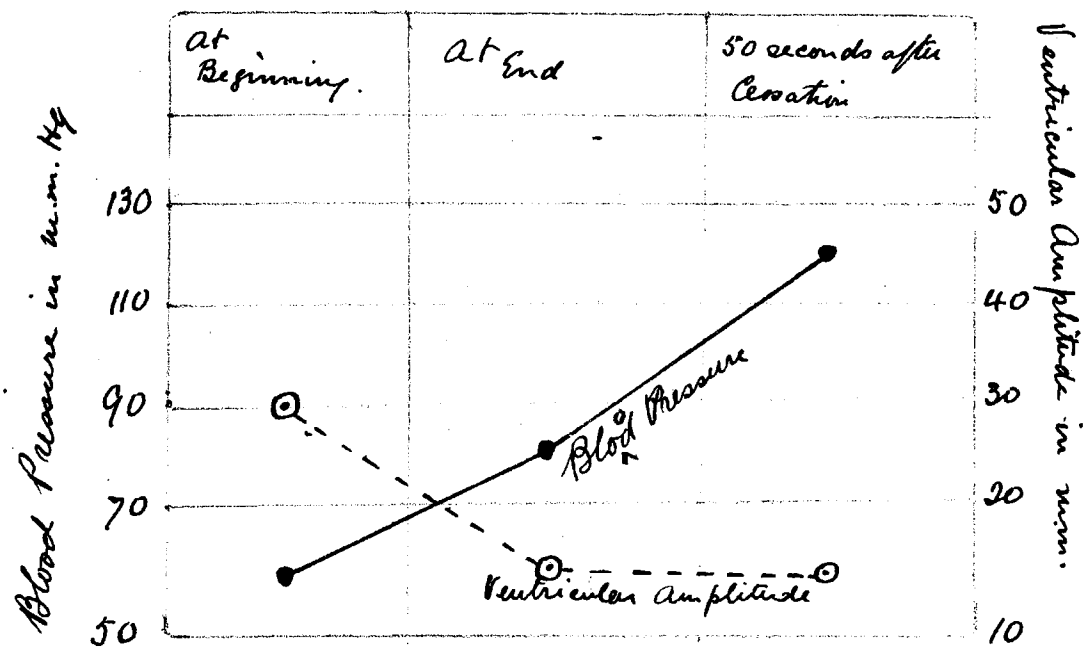
Ventricular amplitude - 15 m.m.

---

50 seconds after cessation of administration of CO_2

Blood Pressure - 120 m.m. Hg.

Ventricular amplitude - 15 m.m.



Protocol II. Deeply under.

Blood Pressure - 43 m.m. Hg.

Ventricular Amplitude - 10 m.m.

4 Litres 12% CO_2 - Air Mixture given.

At end of administration lasting 60 seconds.

Blood Pressure - 25 m.m. Hg.

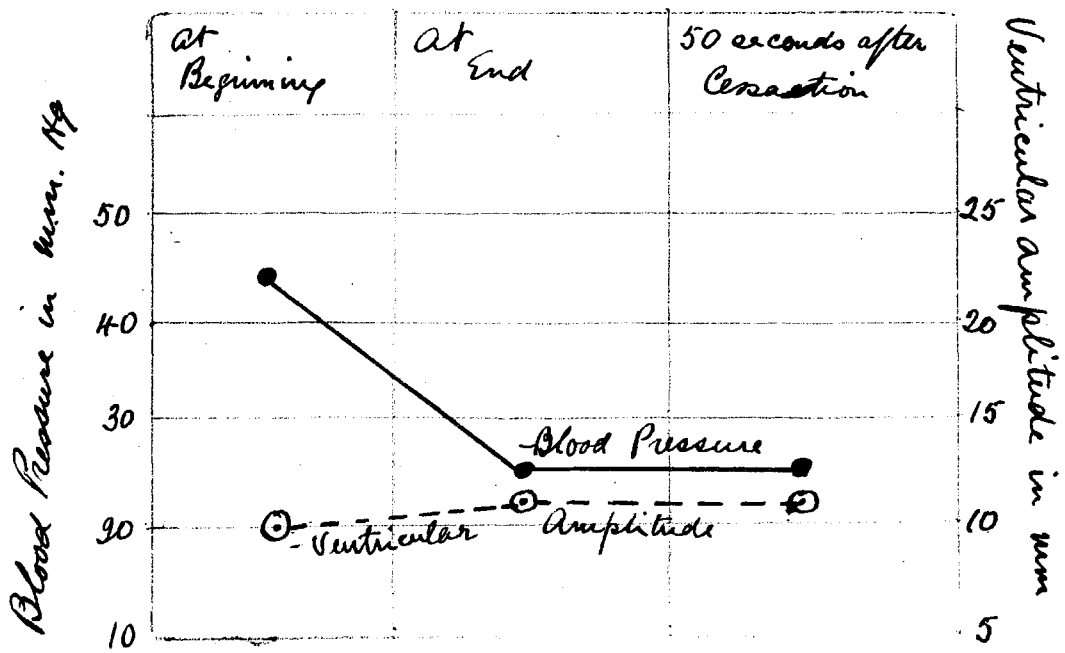
Ventricular Amplitude - 12 m.m.

---

50 seconds after cessation of Administration of CO_2

Blood Pressure - 25 m.m. Hg.

Ventricular amplitude - 12 m.m.



Protocol III. Lightly under.

Blood Pressure - 120 m.m. Hg.

Ventricular Amplitude 15 m.m.

4 Litres of 20% CO_2 - Air Mixture given.

At end of administration lasting 50 seconds.

Blood Pressure - 118 m.m. Hg.

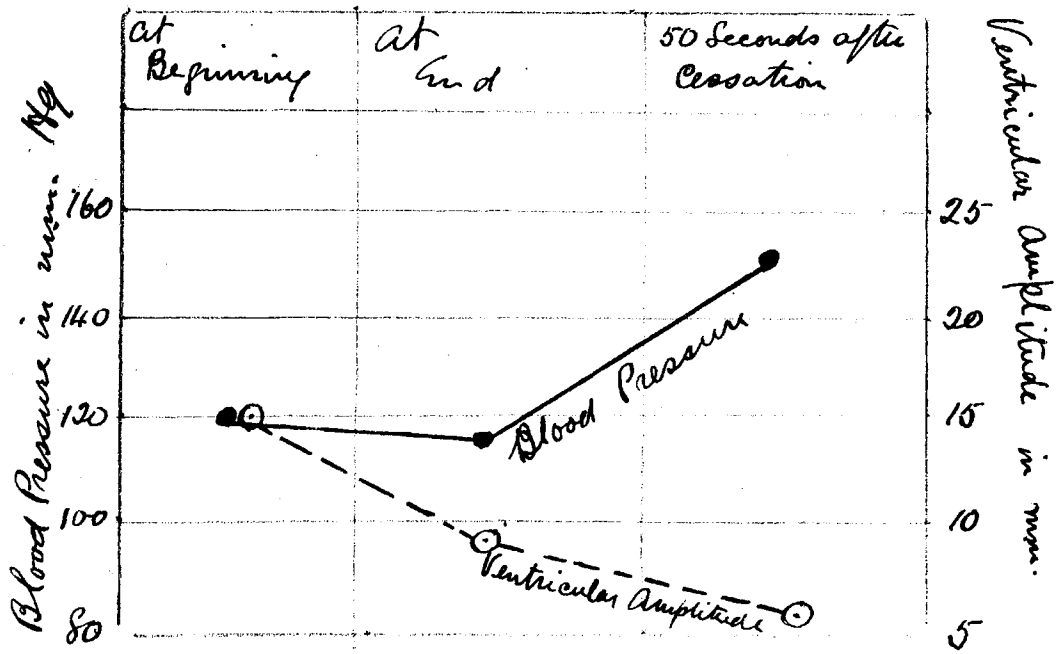
Ventricular Amplitude 8 m.m.

---

50 Seconds after cessation of administration.

Blood Pressure - 150 Hg.

Ventricular Amplitude 6 m.m.



Protocol IV. Deeply under.

Blood Pressure - 45 m.m. Hg.

Ventricular Amplitude - 10 m.m.

4 Litres of 20% CO_2 - Air Mixture given.

At end of administration lasting 50 seconds.

Blood Pressure - 27 m.m. Hg.

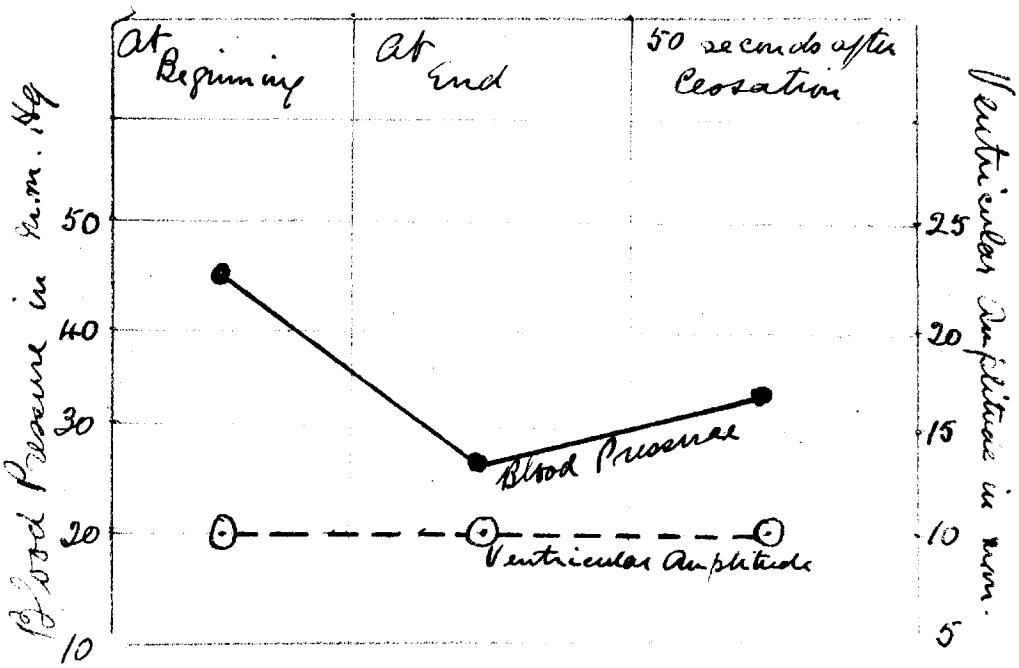
Ventricular Amplitude - 10 m.m.

---

50 Seconds after cessation of administration.

Blood Pressure - 33 m.m. Hg.

Ventricular Amplitude - 10 m.m.



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REPRINT FROM THE

PROCEEDINGS

OF THE

ROYAL SOCIETY OF EDINBURGH.

SESSION 1908-1909.

VOL. XXIX.—PART V.—(No. 26.)

On the Histological Changes in the Liver and Kidney after Chloroform administered by Different Channels.

By **G. Herbert Clark**, M.B., D.P.H.

EDINBURGH:

PUBLISHED BY ROBERT GRANT & SON, 107 PRINCES STREET, AND WILLIAMS & NORGATE, 14 HENRIETTA STREET, COVENT GARDEN, LONDON

MDCCCCIX.

Price One Shilling and Threepence.

XXVI.—On the Histological Changes in the Liver and Kidney after Chloroform administered by Different Channels.

By G. Herbert Clark, M.B., D.P.H. (From the Physiological Laboratory of the University of Glasgow.) (With Three Plates.)

(MS. received April 17, 1909. Read May 3, 1909.)

In a paper published in the *Proceedings* of this Society in 1908, D. Noël Paton (1) showed that chloroform acts very differently upon the metabolism when administered by different channels: that, when given by the respiratory tract, its effect generally is simply to increase the protein metabolism, but that when given by the mouth, it produces a marked disturbance in the distribution of the urinary nitrogen, which he considers to be due to the chloroform acting as an hepatic poison. The action of the drug when administered hypodermically was found to be in the same direction as when given by the mouth. Miss Lindsay in conjunction with D. Noël Paton (2) showed that the rate of elimination varies with the mode of administration, being most rapid when given by the respiratory passages, and slowest when given by the mouth. It was further shown that the chloroform is fixed in the liver to a greater extent when given by the mouth than when given by the respiratory passages. They also, in confirmation of the work of others, recorded the appearance of albumin and of cellular debris and tube casts in the urine, especially after administration by the mouth or hypodermically.

It therefore seemed desirable to study how far the action of chloroform upon the tissues varies with the mode of administration.

Previous Investigations.

Already a very large amount of work upon the action of chloroform upon the tissues has been recorded. An excellent résumé of the literature is given by Stiles and M'Donald (3) in their paper on delayed chloroform poisoning, and only a general statement of the results of previous investigations is necessary.

It appears to be generally recognised that administration of chloroform is often followed by degenerative changes in various tissues, and the majority of writers consider the change to be of the nature of a fatty degeneration. According to some, droplets of oil are to be seen in the blood-vessels.

Fraenkel (4), Marthen (5), and Cohn (6) further describe the appearance of a yellow pigment in the kidneys and liver of patients who died apparently as a consequence of having been anæsthetised with chloroform administered through the respiratory passages.

There is considerable difference of opinion as to the cause of the degenerative change; some authors (Junkers (7) and Strassmann (8)) considering that it is due to the toxic action of the drug on the cells themselves, others to a primary destructive action on the red corpuscles. (Nothnagel (9) and Ostertag (10)).

In the paper referred to above, Stiles and M'Donald describe in detail the post-mortem appearance of tissues removed from a child who died four days after an operation under chloroform anæsthesia.

The changes consisted in extensive degeneration of liver and kidney tissue. In the case of the liver, the cells throughout the organ were markedly changed and vacuolated, but the most complete degeneration appeared to have taken place in the centre of the lobules. The cells contained droplets of oil, which were clearly demonstrated by staining with Sudan iii. Droplets of oil were also found in the hepatic veins. The kidney showed intense fatty degeneration, which was almost universal throughout the organ.

Stiles and M'Donald then made a series of observations upon rabbits, and to obtain a full action of the drug upon the tissues the chloroform was injected subcutaneously.

Here again they found marked fatty changes in the liver cells, most marked in the central and intermediate zones of the lobules. The cells were seen to be occupied by numerous minute droplets which showed no tendency to coalesce, and many in the centre of the lobules were completely disorganised.

In the kidneys the changes were less marked, and varied from cloudy swelling in the cells of the convoluted tubules and ascending loops of Henle to well-marked fatty change in these tubules and the collecting tubules. No fat was observed in the vessels, and it was noted that the glomeruli showed no change. The authors then examined similar tissues obtained from animals which had inhaled chloroform vapour for varying times. They found the changes to be similar to those observed after injection of the drug, but somewhat less marked.

Doyon (13) also describes the histological changes in the liver after chloroform had been administered experimentally by the mouth and hypodermically. In both instances he found necrosis of the liver cells.

Present Investigation.

In carrying out this research the tissues used were for the most part those obtained from the rabbits referred to in the papers by D. Noël Paton and Miss Lindsay.

A. Changes in Vitro.

Before considering the changes which occurred in the body, it was thought advisable to find what was the effect of chloroform upon the tissues when it acted upon them in saline solutions kept at the body temperature. This subject was touched upon by D. Noël Paton in 1894 (11).

In the first instance, 0·75 per cent. sodium chloride solution was used, and a small quantity of pure chloroform was introduced into a bottle filled with the saline, and shaken up with it thoroughly for about five minutes. The solution was then set aside for some time, and the supernatant fluid decanted off. The tissue was removed from a newly killed healthy animal, and having been divided into portions of about $1 \times 1 \times \frac{1}{2}$ c.m., these were immersed for varying times in the solution. As controls, portions of the tissue were fixed immediately in 10 per cent. formol-saline solution, and other fragments of similar size placed in a 0·75 per cent. sodium chloride solution, and left for times similar to those during which the chloroform saline acted. The tissues were kept in an incubator at the constant temperature of 35° C. for periods of $\frac{1}{4}$ hour, $\frac{1}{2}$ hour, 1 hour, $1\frac{1}{2}$ hour, etc., to 24 hours. As the time elapsed, the tissue was removed from the incubator and immediately immersed in a 10 per cent. formol-saline solution. After fixation and hardening, the fragments were cut in paraffin, stained with hæmalum and eosin, and examined. The tissues were also stained by osmic acid, Scharlach rot, and Sudan iii. for fat.

It was found that even the fresh tissue fixed in formol-saline containing 0·75 per cent. of sodium chloride immediately after death shows a slight variation from the normal. The cells look swollen, and their borders are not sharp. It seemed probable, therefore, that the saline solution used was not isotonic with the tissue immersed in it. The solution suggested by Castaigne and Rathery (12) ($\Delta = 0{\cdot}78$), approximately 1·3 per cent. of sodium chloride, was then tried.

Kidney.—In this solution the kidney retains a normal appearance for a considerable length of time, and even after two hours' immersion at a temperature of 35° C. there is but little change in the appearance of the cells. In the later experiments the chloroform was added to this solution.

It was found that necrobiotic changes set in at once in the tissue in

chloroform, but that they do not appear in the control tissue for some time. After five hours the change in the control tissue is quite as great as in the chloroform tissue, and from that time onwards the greatest changes are present in the control tissue. This is due to the action of micro-organisms.

In the kidney the first change which was observed was that the cells of the convoluted tubules seem to lose their definiteness of outline and the protoplasm takes on an appearance like cloudy swelling. The cells appear to be markedly granular, and their free margins rapidly take on a fringed appearance. This is followed by a gradual loss of power of taking on basic stains on the part of the nucleus.

The degenerative changes observed in the tubules, beginning in a cloudy swelling, become more marked as time progresses, until eventually it was difficult to make out the details of structure of the organ. The tubules become filled with granular debris, and little is left beyond the basement membrane mapping out the position which the tubules have occupied.

The glomerular tuft is unaffected in the earlier stages; and, in fact, it is not until after the tissue has been immersed in chloroform-saline solution for three hours that the first marked change appears. This change consists of a slight shrinking of the glomerular tuft away from the capsule of Bowman. In the earlier stages this shrinkage is slight, and leaves a gradually increasing space between the tuft and the capsule, which after longer immersion is found to be occupied by an exudate staining deeply with hæmalum. The same shrinking and exudation appears in the glomeruli of the specimens immersed in saline solution, but some hours later. This confirms *in vitro* the observations of Marthen (5) and Stiles and M'Donald (3).

Liver.—In the case of the liver the influence of the chloroform is well shown, for at an early stage the normally well-defined nucleated liver cells lose their clearness of outline. The finely granular protoplasm becomes coarsely granular, and later becomes broken up and vacuolated. The nuclei soon lose their power of taking on basic stains, and in course of time the cells become so far disintegrated that but little sign of their normal structure persists. After many hours the sections show merely granular debris. The same changes take place in the tissue immersed in saline; but, as in the case of the kidney, this appears after a much longer immersion.

Comparing the whole series of specimens which were examined in these experiments it may be broadly stated that *in vitro* the tissues immersed in chloroform-saline solution show necrobiotic changes at a very much earlier stage than do similar tissues immersed in a similar amount of a pure saline solution of the same concentration.

B. Changes in the Living Animal.

In considering the influence of the drug upon the tissues *in vivo*, two different factors have to be dealt with—the method of administration and the duration of the action of the drug.

Administration by the Respiratory Tract.

In the tissues taken from animals which had chloroform administered through the lungs the amount of change in the liver and kidney was on the whole but small. In some cases, notably those examined some time after anæsthesia, no variation from the normal was observable. The greatest change was in the organs removed from a rabbit which died immediately after the administration. Here the cells lining the ascending and descending tubules of Henle and the convoluted tubules of the kidney showed marked degenerative changes. The liver was much less affected, the cells being in an early stage of albuminous degeneration. It is notable that at the time the animal died the blood contained as much as 77·3 mg. of chloroform per 100 c.c. of blood, and that respiration had stopped during the administration of the drug. The specimens showing the next greatest change were those obtained from an experiment where a small-sized rabbit was anæsthetised for a short time and killed soon after. The blood was found to contain 30·8 mg. chloroform per 100 c.c. The sections of both kidney and liver showed extensive degeneration.

Administration by the Stomach.

When chloroform was administered in oil by the stomach the mortality was very great, and in those animals that survived the administration extensive changes in the organs were found. The kidney tissue had undergone marked degeneration, and in many cases this had gone as far as actual necrosis. The tubules were frequently found to be choked with albuminous debris. In many instances granules which stain bright red with Scharlach rot were observed in the cells and in the debris. The nuclei showed a varying degree of affinity for the basic stain, losing the power to take on the stain as degeneration advances. The glomeruli were in no instance in an advanced stage of degeneration, signs of congestion alone being present. This observation is in accordance with the results obtained by the experiments *in vitro*.

The degree of change in the kidney varies greatly with the length of time after the chloroform is administered. In cases where the animal was

killed within a few hours of this administration, degeneration had not advanced very far. Three hours after the drug had been given the cells showed a considerable degree of cloudy swelling, and here and there there were signs of desquamation in the ascending and descending tubules of Henle.

In a specimen taken at 5½ hours the degeneration had advanced greatly, and the tubules contained a great deal of albuminous material. The cells were frequently found to be vacuolated, and the nuclei had taken on the hæmalum stain badly. When the animal recovered, the kidney tissues apparently began to repair after arriving at this point of degeneration. In specimens taken from animals which were killed two or three days later, apparently recovering from the effects of the drug, the changes were never found to be more marked than those described. On the other hand, when the animal died overnight after the administration or was killed when obviously dying, the kidney was found to be rapidly losing all signs of its original structure; the cells lining the tubules were frequently lost altogether, nothing being left but the basement membrane. Where the cells were still apparent, the nuclei were stained badly and the tubules were choked with debris.

Here and there throughout the organ, particularly in one or two specimens, masses of blood were observed, apparently between the tubules and not in the vessels (figs. 1 and 2). Where this was observed, the cells lining the tubules adjacent to the blood were frequently found to contain small dark-coloured granules similar to those described by Fraenkel (4), Marthen (5), and Cohn (6). These were not observed in any other position in the kidney.

In the liver the degree of change was also found to vary with the length of time after the administration of the drug, and also with the progress towards recovery of the animal.

Examined three hours after administration, the cells at the periphery of the lobules showed but little change—at most a slight degree of cloudy swelling. The cells in the centre of the lobule, on the other hand, had undergone a granular change, and the nuclei had begun to lose their power of taking on the stain.

After five hours this was still more marked; and an hour and a half later some of the cells in the centre of the lobule had completely broken down, leaving granular debris in place of the cells.

When the animal showed evidence of recovering from the effects of the drug, no further change in appearance of the tissues was observed. When the animal was found dead in the morning after the administration, or

showed signs of dying at an early date and was killed in consequence, the degree of change was very much greater. The centre of each lobule was found to be occupied by a granular mass showing neither nuclei nor any appearance of liver tissue. The intermediate zone was frequently also affected, and in the worst cases no sign of liver tissue was seen except a layer of two or three cells thick at the periphery of the lobules (fig. 3). In the granular debris a number of granules staining red with Scharlach rot were seen.

The degenerative change in the liver in the animals where the chloroform was administered by the stomach is generally very great indeed, and in all cases appears much more complete than the change in the kidney. This is possibly due to the action of the chloroform "anchoring" itself to the liver cells.

Administration Subcutaneously.

When chloroform was given in the form of a subcutaneous injection, the mortality among the animals was very great, and a large proportion died during the night after the injection.

On the other hand, the animals that recovered appeared to be quite active a day or two later.

Histologically, the changes in the organs are very similar to those detailed above, the difference being one of degree.

In the kidney, after four hours very little degeneration seems to occur, a slight degree of cloudy swelling being apparent. After five hours, some vacuolation was seen in one of the specimens examined. In one animal killed some hours later, and in a dying condition, the kidney showed an appearance comparable with that observed in some of the worst cases after administration by the stomach. The ascending and descending tubules, the convoluted tubules, and more markedly the collecting tubules showed little or no sign of cellular lining. They were choked and frequently distended with albuminous debris. In all the cases, however, the glomeruli retained an appearance approximating to the normal.

In the animals which died, the appearance of the kidney was similar to those just described. Parts showed tubules denuded of their epithelium, and other parts showed cells in an advanced state of degeneration (fig. 4). Generally there was evidence of congestion of the organ, and occasionally dark granules were observed similar to those referred to above.

In the case of the liver there was generally a slight necrosis in the centre of the lobule even a very few hours after the administration, and as

time advanced this increased in amount (fig. 5). In the worst cases, where the animal was found dead or was killed in a dying condition, the state of the organ was like that described under administration by the stomach, viz., the organ was a honeycomb of cheesy material showing very little sign of the original liver structure.

Summary.

1. When kidney or liver tissue is immersed in a saline solution containing chloroform, degenerative changes take place similar to the normal necrobiotic changes but very much more rapid. In the case of the kidney the glomeruli are not affected for a very considerable time.

2. When chloroform is administered through the respiratory passages a considerable degree of degeneration is only occasionally found in the kidney and liver cells. It is more marked in some cases than in others where a similar amount of chloroform was given to animals of a similar size. This may be associated with the very varying rate at which the drug is eliminated, as shown by Miss Lindsay (*loc. cit.*). The degree of change in the liver was never great. In the kidney there is frequently cloudy swelling, and occasionally desquamation of the epithelium of the ascending and descending tubules.

3. Where the drug is given by the stomach the mortality is great and the changes observed in the organs are marked. In all cases there is evidence of the toxic action of the drug. In the animals most affected, the structure of the liver is almost entirely lost, nothing remaining of the lobules but a shell of liver cells enclosing a cheesy debris.

In the kidney the drug acts in a similar way, the degree of degeneration being somewhat less than in the liver.

The glomeruli are but little affected even in the worst cases.

4. When the drug is given hypodermically the changes are similar to those observed when the drug is given by the stomach. The liver is again more affected than the kidney.

On the whole, however, chloroform does not appear to be quite so destructive to the liver tissue when administered in this form.

5. The marked action of the drug upon the liver, whether administered by the stomach or hypodermically, is probably accounted for by the "anchoring" action referred to by D. Noël Paton (1). It would be interesting to know if there is evidence of a similar action on the part of the kidney cells to account for the extensive degenerative change frequently observed there.

6. The result of these observations helps to explain the different effects of chloroform on hepatic metabolism. When given by the respiratory passages it is rapidly eliminated, produces no marked histological changes, and the metabolic disturbances are slight; but when given by the mouth and hypodermically it is more slowly eliminated, has more time to produce its toxic action, and the metabolic disturbances are pronounced.

In a future paper the action of chloroform upon the blood corpuscles will be dealt with.

A grant was received from the Carnegie Trust to defray the expenses of this research.

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(*Issued separately July* 9, 1909.)

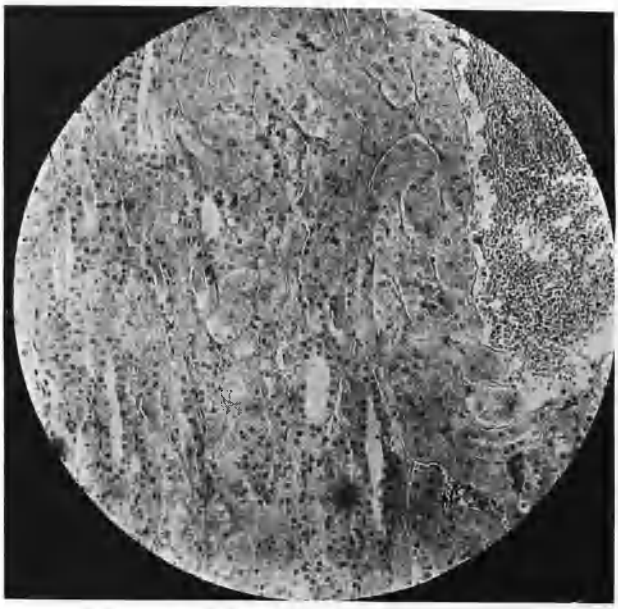

FIG. 1.—Kidney from rabbit, No. 9a of the series, which had 1cc of chloroform administered in oil by the stomach. To the right is seen a quantity of extra-vascular blood. The cells lining the tubules are in various states of degeneration. The nuclei, as a whole, are badly stained. ×150.

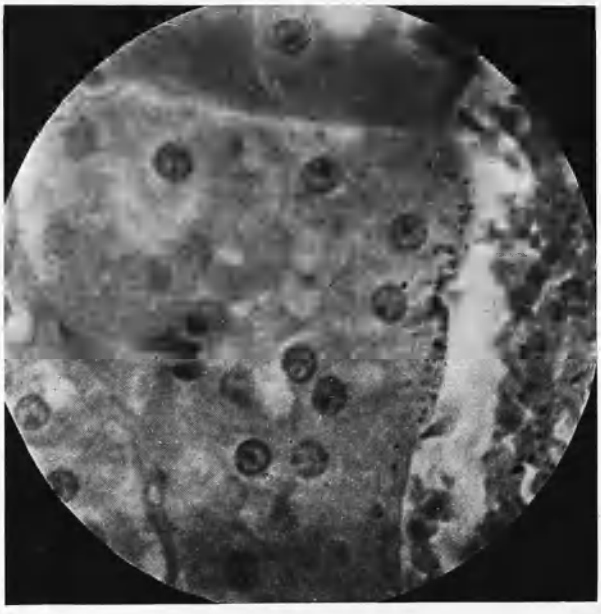

FIG. 2.—High power view of the tubules adjacent to the extra-vascular blood. The granules referred to in the text are well shown. ×800.

DR G. HERBERT CLARK. [Plate I.

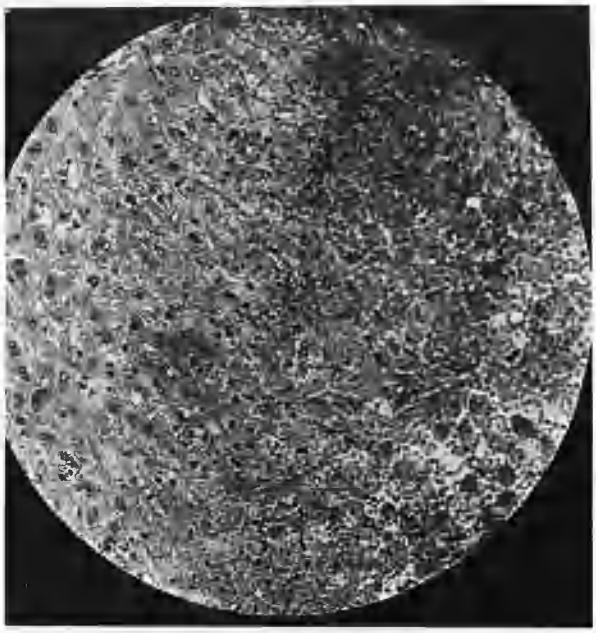

FIG. 3.—Liver from the same rabbit. To the left the centre of the lobule is seen to be completely broken down, while to the right the cells at the periphery appear almost normal. ×150.

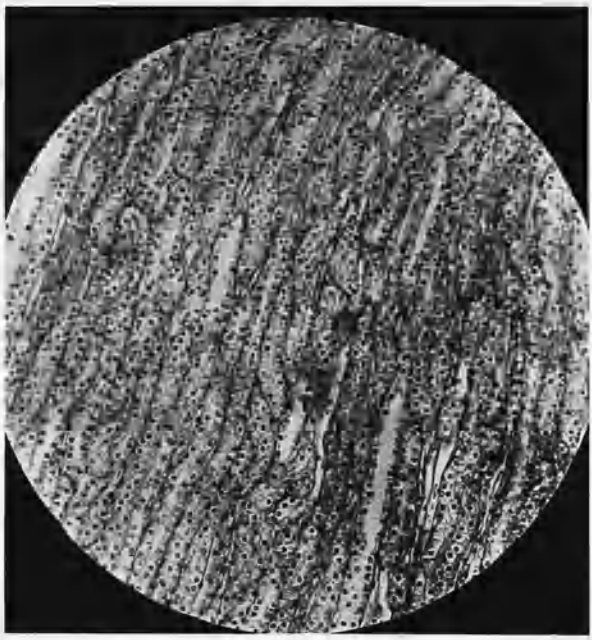

FIG. 4.—Medulla of kidney from rabbit, No. 33, which received an injection of 1cc of chloroform subcutaneously. A considerable degree of degeneration is observable, and the nuclei are losing the power of taking on the basic stain. ×150.

DR G. HERBERT CLARK. [Plate II.

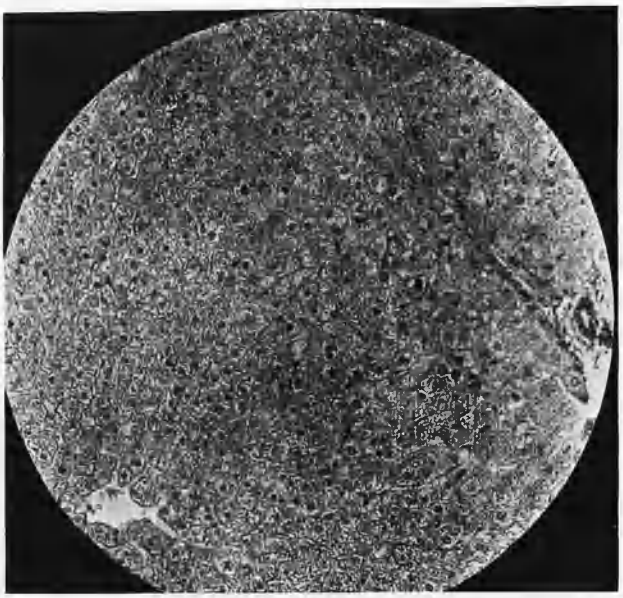

FIG. 5.—Liver from the same animal. The centre of the lobule shows cells considerably degenerated and even vacuolated. The periphery—to the right—shows cells which are almost normal in appearance. ×150.

2

THE

INFLUENCE OF CHLOROFORM WHEN REPEATEDLY ADMINISTERED IN SMALL DOSES

BY

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(From the Physiological Laboratory, University of Glasgow)

Reprinted from THE LANCET, January 21, 1911

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THE INFLUENCE OF CHLOROFORM WHEN REPEATEDLY ADMINISTERED IN SMALL DOSES.

THE very striking results obtained when administering a single large dose of chloroform and referred to at length in a paper published in 1908 (1) * suggested to the author that a very much smaller dose might have some influence on the tissues of an animal, particularly when the small dose was repeated daily.

Sir James Crichton-Browne in 1907 (2) called attention to the habit in certain districts of taking lozenges and jujubes containing chloroform, and pointed out that samples of the lozenges contained as much as 2·9 per cent. of chloroform. He pointed out that these lozenges are taken in considerable quantity and over long periods, and he expressed the opinion that such repeated doses must be harmful. The repeated administration of small doses of this drug by the stomach to lower animals is therefore of practical as well as of scientific interest.

In the series of experiments undertaken rabbits were used throughout and the chloroform was administered as in the paper referred to above in one of three different ways—either by inhalation, by the mouth, or subcutaneously. The weight of the animal was taken day by day, and after death in many cases the weight of the heart was taken, and also the weight of the spleen. In all cases portions of liver, spleen, kidney, and heart were examined for fat and also stained and examined for degenerative changes. In the first experiments of the series the animals which were injected with $CHCl_3$ had 0·2 c.c. on two consecutive days, and two days were allowed to elapse before this procedure was repeated. This method proved so rapidly fatal that in the sub-

* The parenthetical figures throughout the paper refer to the bibliography at the end.

sequent experiments 0·1 c.c. of $CHCl_3$ was injected daily. In the cases where the rabbits received the $CHCl_3$ in the form of an inhalation they were rapidly anæsthetised and then kept lightly anæsthetised for 15 minutes. In the first experiments of this series this was done daily, but as all the animals used died while under the anæsthetic after a very few days the procedure was modified and the anæsthetic given on alternate days. When $CHCl_3$ was given by the stomach it was found that $CHCl_3$ water was easily tolerated by the rabbits and consequently 40 c.c. of this solution were given daily. This contained 0·08 gramme of chloroform. In some cases the rabbits lapped up the fluid without trouble, but in most instances a stomach tube was passed under light ether anæsthesia and the $CHCl_3$ water administered through this. Considering the results of the experiments throughout the series the effect of the $CHCl_3$ administered in the various ways upon the well-being of the animal may be studied by an examination of the weights from day to day. The weights are given in grammes.

TABLE I.—*Administration by Inhalation.*

No.	Day.									
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.
6	1470	1450	—	—	—	—	—	—	—	—
7	950	920	860	770	—	—	—	—	—	—
8	2250	2250	2120	—	—	—	—	—	—	—
12	1850	1725	1750	1780	1880	1855	1850	1870	1855	1750
20	2300	2275	2270	2270	2250	2200	2400	—	—	—
22	2050	1975	1950	1900	1850	1850	1800	1750	1700	1625*
24	2250	2170	2250	2150	2250	2250	2100	2150	—	—
26	1600	1600	1700	1750	1750	1750	1700	—	—	—
27	1650	1600	1550	1700	1750	—	—	—	—	—
30	2050	1950	1800	1670	1600	—	—	—	—	—
32	2370	2270	2260	2250	2300	2100	2050	1950	1925	1950†
36	1600	1550	1550	1550	1600	1600	—	—	—	—

* Survived for one month. Weight at death 1400 grammes.
† Survived for two months. Weight at death 1840 grammes.

Where the administration took place daily the weight fell fairly rapidly. In the case of rabbit No. 7 the weight had fallen nearly 200 grammes in four days. Where the administration was upon alternate days there was in most cases but little change. The daily rise or fall was similar to what was observed in control rabbits. In a few cases, however, a marked fall in weight took place—e.g., rabbits Nos. 22, 30, and 32. The rabbits took food well, and some lived for a long while, having the chloroform administered as described —e.g., No. 22 lived for more than a month and No. 32 for nearly two months.

TABLE II.—*Administration by Subcutaneous Injection.*

No.	Day.											
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.
1	*1150*	*1150*	1100	1050	1070	*1075*	*1100*	1050	1070	1000	—	—
2	*2000*	1950	1890	*1935*	*1900*	—	—	—	—	—	—	—
3	*1550*	1550	1520	*1470*	*1550*	1450	1430	1450	—	—	—	—
	Daily Injections of 0·1 c.c.											
4	1700	1680	—	—	—	—	—	—	—	—	—	—
5	1430	1400	—	—	—	—	—	—	—	—	—	—
13	2400	2450	2430	2230	—	—	—	—	—	—	—	—
16	2700	2630	2520	2625	2500	2470	2400	2310	2220	2180	2150	2130
19	1700	1680	1700	—	—	—	—	—	—	—	—	—
21	3100	3050	3050	3000	3050	3020	2975	2930	2900	2850	1 month	2725
25	1650	1550	1470	1450	1450	1350	1300	1350	1300	1375	1350	1250
28	1500	1400	—	—	—	—	—	—	—	—	—	—
29	1150	1150	1150	1150	1100	1060	—	—	—	—	—	—
31	2450	2420	2280	2250	2350	2450	2300	2150	2100	1950	26 days	1550
37	1325	1300	1200	1100	1200	1200	1150	—	—	—	—	—

In the first three experiments cited above 0·2 c.c. of the anæsthetic was injected on the days signified by italic figures, and it will be noted that the effect of the injections is to lower the weight on the day or days following. This

effect is very much more marked in the nine experiments of the second series when the chloroform was given daily until the animal died. Here a considerable fall in weight is shown in some of the animals. In rabbit No. 21 there is a loss of 720 grammes in a month, and in rabbit No. 31 a loss of 1100 grammes in a somewhat shorter time. In many of the animals the loss in weight is very slow at first, but as time progresses it becomes much more rapid. The effect upon weight of administering the chloroform in this way is much more marked than when the drug is administered by inhalation on alternate days, but not so rapidly fatal as where it is given daily in that manner.

TABLE III.—*Administration by the Stomach.*

No.	Day.											
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.
9	2380	2320	2200	2250	2250	2160	2210	2175	—	—	—	—
14	2300	2350	2380	2380	2240	—	—	—	—	—	—	—
15	2150	2025	1975	2050	2150	1950	1925	1900	1940	1875	1870	1870
18	2400	2520	2500	2550	2450	2300	2150	4 months later 1350				
23	1700	1700	1750	1700	1650	1550	1500	1500	1450	—	1 mnth 1620	
33	2100	2000	2000	1900	1900	1800	1700	1650	1600	1550	1475	—

The amount of $CHCl_3$ given by this method was somewhat smaller than that given in the two previous series, being about 0·08 gramme per diem, but this amount, small as it is (about the same as the $CHCl_3$ content of one lozenge as described by Crichton-Browne (2)), caused a progressive diminution in weight in the animals. In all these cases the diminution in weight was marked, with the exception of No. 14, where a rise in weight was found. The death of the animal was, however, preceded by a very considerable fall. In No. 18 the rabbit tolerated a daily dose for over four months, but it is significant that in that time the animal had lost nearly 50 per cent. of its original weight—or rather more than a kilogramme.

Histological Changes.

Concurrently with the alteration in body weight in the animals under experiment histological changes in the organs and tissues examined were found. These changes were for the most part similar to those already described by Bandler (3), Heintz (4), and Ajello (5), and later by Doyon (6) and Billet (7).

Administration by Inhalation.

As in the previous series of experiments (1) the kidney and liver suffered considerably when chloroform was administered in this way. The degree of degeneration, however, varied in the different cases, and the difference in degree was not always dependent upon the number of administrations, nor was the change equally marked in the kidney and liver. For example, in the kidney the greatest alteration from the normal was found in animals No. 22, which survived for 31 days, and No. 36, which only survived for six days. The liver in No. 22 was also very badly degenerated, whereas that of No. 36 was not markedly changed. On the other hand, the liver in rabbit No. 30, which lived for five days, showed marked degenerative changes. The cells were coarsely granular and vacuolated; the nuclei took the basic stain badly and fat was abundant. On the whole, it may be stated that the degeneration in the kidney was the most marked feature of administration in this way, and that the kidney was invariably the seat of much change. A point of interest lies in the fact that most of the specimens showed that the glomerular tuft had shrunk away from its capsule—a phenomenon which I showed in a previous contribution was only present after very great changes had taken place in the organ. The liver suffered most in those cases which survived for a considerable time, whereas those animals which succumbed at an early date showed generally a much smaller degree of change. Fat was detected in all instances, the stain used being Scharlach R. The fat was almost always abundant, and generally involved the cells at the centre of the lobule first and extended outwards subsequently.

The spleens of all the animals were examined and the sinuses were invariably found to be engorged with blood. A considerable quantity of an orange-coloured pigment was

generally present, and in most instances enormous phagocytes were abundant, the phagocytes being distended with red corpuscles and often with pigment. The weight of the spleen varied considerably. Four control animals yielded an average weight of 0·78 gramme, each of the animals weighing 2000 grammes or over.

TABLE IV.—*Weight of the Spleen in Animals Anæsthetised.*

No.	Original weight of animal.	No. of days.	Weight of spleen.
12	1850 grammes.	20	2 grammes.
20	2300 ,,	7	0·9 gramme.
22	2050 ,,	31	1·2 grammes.
24	2250 ,,	8	1·95 ,,
26	1600 ,,	7	0·6 gramme.
27	1650 ,,	5	1·4 grammes.
30	2050 ,,	5	0·72 gramme.
32	2370 ,,	65	1·2 grammes.
36	1600 ,,	6	1·2 ,,

The average weight of the animals was 1900 grammes, while the average weight of the spleens was 1·24. A large increase of iron, as demonstrated by the relative amount shown by the ferrocyanide HCl stain on the sections, was found in all cases.

In examining the animals after death it was frequently found that the heart muscle appeared flabby and often the walls seemed unusually thin. These organs were therefore fixed and examined for histological changes. The heart muscle stained unequally; some parts appeared quite normal, while other areas showed a granular appearance quite unlike the normal; the transverse striations were lost and the fibres were often segmented. The nuclei in these parts were poorly stained and in some specimens little was left but a mere indication of the position which they occupied. A large number of the cells were vacuolated. This appearance is referred to by Ungar and Junkers (8), and by Heintz (4). The granular and broken-down appearance was very marked in

some of the hearts examined, notably in Nos. 12, 20, 22, and 24, but not so marked in No. 32, where the animal survived for 65 days.

It may be noted here that all the animals referred to above died while under the anæsthetic, either while the drug was being administered or within five minutes after stopping the administration.

Administration by Subcutaneous Injection.

In the cases of animals Nos. 1, 2, and 3 chloroform was given in doses of 0·2 of a cubic centimetre, and the doses were given irregularly, as shown in Table II. The results in these cases are therefore not strictly comparable with those obtained by daily administration of 0·1 c.c., but they are included here as they illustrate the points to be brought out in this paper.

The animals referred to other than the above received 0·1 c.c. hypodermically each day. The injection appeared to have no immediate effect, and the rabbits so treated were as active after as they were before the administration. Of those which were injected with the larger amount one died after four and two after three administrations, so that the drug was rapidly fatal. Those rabbits which were injected with only 0·1 c.c. lived for longer periods, the average being 11 days. The longest period a rabbit lived during the administration was 30 days and the shortest two days.

The organ which appeared most affected histologically was the liver, and here the changes were comparable with those referred to in a previous paper (1). In one instance, where the rabbit survived for 30 days, the liver was found to be of an orange colour, and contained masses of an orange- or yellow-coloured pigment like that observed in the spleen, and similar to that described by Fraenkel (9), Marthen (10), and Cohn (11). This pigment gave a positive stain for iron. There did not appear to be atrophy. In a few of the livers examined, particularly when taken from animals which had lived for some time, a considerable amount of white fibrous tissue was found between the lobules, as described by Fiessinger (12). The kidneys also generally showed some degree of degenerative change, although in many instances but little alteration from the normal was observed. Congestion and hæmorrhage were generally found, and in the kidneys taken from the animals which were injected with the

larger dose of the drug the degeneration in the convoluted as well as the straight tubules was very marked. Frequently the tubules were packed with débris and desquamated epithelium. Where the degeneration was far advanced fat was always found in the degenerated cells. On the whole, however, the kidney appeared to be affected to a somewhat slighter degree in this method of administration.

The spleens showed much the same condition as did those in Table IV. The amount of iron present as shown by the ferrocyanide HCl method of staining was very great, and in No. 19 when the animal was killed after three administrations fully half of the spleen section stained green by this method of staining. The large phagocytes referred to above were also present here in large numbers.

TABLE V.—*The Weights of the Spleens and other Particulars.*

No.	Original weight of animal.	No. of days.	Weight of spleen.
16	2700 grammes.	13	1·5 grammes.
21	3100 ,,	30	1·85 ,,
25	1650 ,,	15	0·67 gramme.
28	1500 ,,	3	1·2 grammes.
31	2450 ,,	26	1·5 ,,
37	1325 ,,	7	1·5 ,,
Average...	2121 ,,	16	1·37 ,,

Thus the average weight of the spleens was 1·37 grammes, as compared with an average weight of 0·78 gramme in the control animals. The hearts generally showed degenerative change, although this was not always marked. Granular cells with loss of the cross-striped appearance were common, and in most cases the nuclei stained irregularly; sometimes the peripheral part of the cardiac muscle appeared to have suffered most, at other times the central parts.

Fat was not observed in any of the hearts examined for it, therein differing from the findings of Fraenkel (9) and supporting Heintz (4).

Administration by the Stomach.

The method of administration adopted here was not by any means so rapidly fatal as either of the other methods, contrasting in this with the administration of larger doses in olive oil as detailed in a previous paper, where this was the most fatal method of administration. The average length of life during the administration was 16 days, not taking into account the animal that lived for four and a half months receiving an administration each day. As might also be expected, the liver suffered most of any organ when the chloroform was administered in this way. The amount of degenerative change was great in all cases, but was of a different type in those animals which lived for some time to that in those where death took place in a few days. In those dying soon the cells showed albuminous degeneration, vacuolation, and on two occasions all that could be made out of the liver cells was a kind of network outlining spaces which contained a poorly staining central nucleus surrounded by a small amount of coarsely granular protoplasm. In rabbit No. 18, which lived for more than four months, there was a very great deal of fibrous tissue throughout the liver. Large openings were found irregularly distributed throughout the specimen, and the altered cells showed no sign of definite arrangement.

The greatest degenerative change was observed in No. 38, where the centres of the lobules had lost all trace of their cellular components and were made up of a cheesy mass containing numerous dark granules. Abundance of fat was always found.

The kidneys had on the whole suffered comparatively slightly, although in Nos. 14 and 33 the cells in places were badly degenerated, showing but little sign of the original structure. In No. 33 the glomerular tufts were shrunken, and in a few places in the cortex as well as in the medulla the cells of the tubules were entirely lost. There was rarely congestion and no hæmorrhages were found.

In the spleen the sinuses were found distended with blood, and pigment was present. In Nos. 14, 15, and 23 the amount of iron shown by the ferrocyanide HCl stain was but small, although in 18 and 33 the amount was very great.

The large phagocytes were numerous in those specimens which contained much pigment.

TABLE VI.—*The Weights of the Spleens and other Particulars.*

No.	Original weight of animal.	No. of days.	Weight of spleen.
14	2300 grammes.	5	1·2 grammes.
15	2150 ,,	14	0·3 gramme.
18	2400 ,,	140	1·5 grammes.
23	1700 ,,	30	0·95 gramme.
33	2100 ,,	11	0·8 ,,

The average weight of the animal was 2130 grammes, while the average weight of the spleens was 0·95 gramme, the latter average being 0·17 gramme above the normal. The hearts showed very little variation from the normal, and the cross striping was seen plainly in all the specimens examined.

Consideration of Results.

In reviewing the results of the experiments detailed above, it appears to be proved that given in the form of small doses frequently repeated chloroform is a much more dangerous drug than when given in a single much larger dose. The first doses given appear to lower the vitality of the tissues, so that the later doses have a more marked action.

Idiosyncrasy or a number of undetermined factors appear to influence the action of chloroform on animals, and we find enormous variations between the shortest time and the longest time an animal lived during the administration.

In all the animals examined the liver appeared to have suffered greatly, although, as was to be expected, the most marked degeneration was found where the anæsthetic was given through the stomach. When the drug was inhaled the kidneys seem to have suffered more uniformly than in the other cases, and although the degree of degeneration was not always great, the difference in degree between kidney and

liver degeneration was not nearly as marked as when the chloroform was given in either of the other ways.

Very great changes were seen almost uniformly in the spleen even in the animals that died in a few days. The evidence of extensive hæmolysis was most marked. The large-sized phagocytes in many cases distended with dead red corpuscles and in other cases containing a pigment which gave a positive stain for iron, the masses of such pigment apparently lying loose in the spleen, and the large size and weight of the organ all demonstrated its increased activity (Paton and Goodall) (14). The organs in which this increased activity was least manifest were those removed from the rabbits which received the administration through the stomach much diluted with water. Here the average size of the spleen was small and the weights corresponded. The average weight was only 0·17 gramme above the normal, whereas in the other experiments the increase was 0·46 gramme when chloroform was inhaled and 0·59 gramme where it was injected.

When chloroform was inhaled it seemed to have a more marked action on the heart muscle than when it was given through the stomach or injected. Fat was not detected in the hearts examined.

In connexion with the administration by the stomach, it is of interest to note that the amount of chloroform given in each administration was comparable with the quantity described by Sir James Crichton-Browne (2) as being present in one linseed, liquorice, and chlorodyne lozenge. The average weight of a full-grown rabbit is about 2 kilogrammes, while 70 kilogrammes represents the weight of a full-grown man. Thus 30 to 40 of these lozenges per diem in a man gives the same proportion of chloroform to body weight as in the experiments, and correspondingly less in a woman or a child. Many rabbits are very susceptible to the action of chloroform, but even in the one example where the animal lived for a long while the liver changes were of a very striking description. The lesions found in rabbits closely resemble those reported in fatal human cases of chloroform poisoning by Stiles and McDonald (15) and others.

The mortality among rabbits which have been anæsthetised through the respiratory passages and the changes in heart, liver, and kidney found after death emphasise the danger of repeated administration by this method, even when the anæsthetic is only exhibited for very short periods.

Summary.

1. Chloroform repeatedly administered by the respiratory passages, subcutaneously, and by the stomach in small doses rapidly kills rabbits.

2. The liver shows degeneration of the cells sometimes so marked that the whole centre of the lobule is broken down into débris. The cells in the centre of the lobule are early affected, those further out later. Fat is always present, generally in large quantities.

3. The kidney suffers to some extent, but relatively more when the chloroform is inhaled than when injected or given by the stomach. Fat is occasionally found in degenerated cells.

4. The spleen shows intense congestion, the sinuses being packed with red blood corpuscles. Along with the red corpuscles an orange-coloured pigment is generally present which reacts to the stain for iron. A large number of very large phagocytes are present in most cases. The average weight of the spleen was 0·46 gramme heavier than the controls when chloroform was inhaled, and 0·59 gramme and 0·17 gramme heavier when injected and when given by the stomach respectively.

5. Degenerative changes were observed in the cardiac muscle. Fat was not observed in any of hearts examined.

(A grant was received from the Carnegie Trust to defray the expenses of this research.)

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Glasgow.

Reprinted from THE LANCET, *July* 27, 1912.

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THE DISTRIBUTION OF CHLOROFORM IN THE BLOOD.

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Buckmaster and Gardner[1] have shown that in chloroform anæsthesia the bulk of the chloroform is carried by the red corpuscles. Pohl[3] estimated the proportion in the corpuscles as two to four times that present in the serum. Nicloux[4] by a more accurate method estimated the amount in corpuscles as about 88 and in the plasma 12 per 100 parts of chloroform. The enormous difference in time of absorption and of elimination, and the very much more marked effects on the tissues of chloroform when given subcutaneously, suggested to us that the chloroform might be differently combined in the two cases. By inhalation the greater part of the chloroform combines loosely with the corpuscles and is rapidly absorbed and rapidly eliminated. A small moiety is sometimes found according to Noel Paton and Miss Lindsay[5] still present in the blood some hours after the anæsthetic, particularly when the animal anæsthetised has been confined in a cage after the administration. This might represent a remnant of the 12 per cent. in the plasma. When given subcutaneously the much slower absorption and elimination, and the consequent greater effect on the tissues, might be due to a firmer combination of the chloroform with some part of the serum—say, the proteins, as suggested by Moore and Roaf.[2] That the drug is differently fixed in the two cases is further suggested by the fact that a prolonged administration of chloroform by inhalation has very little effect on the liver and kidney tissues, whereas the administration of a very small amount hypodermically causes injury to the cells of these organs.

In carrying out the experiments the same procedure was observed in all cases. The rabbits were weighed, and then where they were anæsthetised by inhalation they were kept just under for one hour. They were then killed and rapidly bled an amount of 15 per cent. solution of potassium oxalate sufficient to prevent coagulation of the blood. The blood mixture was then centrifugalised for three hours, and the corpuscles and plasma were separately estimated for chloroform. The method of Nicloux[3] was used throughout the estimation.

The time taken for centrifugalising—three hours—is the same as was adopted by Nicloux, and yielded more constant results than any shorter time. As an objection against such a lengthy separation may be argued that both corpuscles and chloroform would be driven to the bottom of the tube, and thus the proportion in corpuscles would rise with prolonged separation. This, as a matter of fact, is the case, but may also be due to the separation being more complete. Where the centrifugalising has gone on for one hour the corpuscles plus chloroform are mixed with a certain amount of plasma plus its chloroform ; in the latter case the chloroform content is small and tends to reduce the proportion of chloroform in the mass. At the end of three hours the amount of plasma and chloroform with the corpuscles is very small indeed, and is negligible. In any case, the same procedure was adopted with both inhalation and injection specimens, so that the results are comparable.

Where chloroform was given subcutaneously amounts varying from 1 c.c. to 3 c.c. were given, and the animals were killed and bled as above two to three hours later. The amount of chloroform in the blood approaches a maximum two to four hours after the administration.[5] The results are :—

1. *By Inhalation.*

Rabbit D.—Weight, 1800 grm. Chloroform inhaled for one hour. 35 c.c. of blood taken with 1 c.c. of 15 per cent. potassium oxalate ; centrifugalised for three hours. Total amount recovered, 18·13 mg. chloroform. Corpuscles, 90·8 per cent. ; plasma, 9·2 per cent. of total chloroform present.

Rabbit M.—Weight, 2200 grm. Chloroform inhaled for 45 minutes. 58 c.c. of blood taken with 2 c.c. of 15 per cent. potassium oxalate ; centrifugalised for three hours. Total amount recovered, 26·56 mg. chloroform. Corpuscles, 90·2 her cent. ; plasma, 9·8 per cent. of total chloroform present.

Rabbit P.—Weight, 1850 grm. Chloroform inhaled for one hour. 35 c.c. blood with 5 c.c. potassium oxalate ; centrifugalised for three hours. Total amount recovered, 6·3 mg. chloroform. Corpuscles, 88·8 per cent. ; plasma, 11·2 per cent. of total chloroform present.

Rabbit R.—Weight, 1900 grm. Chloroform inhaled for one hour. 38 c.c. of blood with 5 c.c. of potassium oxalate; centrifugalised for two and a half hours. Total chloroform recovered, 16·45 mg. Corpuscles, 88·9 per cent.; plasma, 11·1 per cent. of total chloroform present.

Rabbit S.—Weight, 2400 grm. Chloroform inhaled for one hour. 45 c.c. of blood with 5 c.c. of potassium oxalate; centrifugalised three hours. Total amount recovered, 14·84 mg. chloroform. Corpuscles, 87·6 per cent.; plasma, 12·4 per cent. of total chloroform present.

Rabbit Z.—Weight, 1800 grm. Chloroform inhaled for one hour. 40 c.c. of blood with 5 c.c. of potassium oxalate; centrifugalised for two hours. Total amount recovered, 14·84 mg. chloroform. Corpuscles, 85·2 per cent.; plasma, 14·8 per cent. of total chloroform present.

Average distribution in six rabbits: Corpuscles, 88·6 per cent.; plasma, 11·4 per cent. of total chloroform present.

2. *By Injection.*

Rabbit C.—Weight, 2400 grm. 1 c.c. injected; animal killed four hours later. 40 c.c. blood taken with 1 c.c. of potassium oxalate; centrifugalised three hours. Total amount recovered 7·9 mg. chloroform. Corpuscles, 81·6 per cent.; plasma, 18·4 per cent. of total chloroform recovered.

Rabbit G.—Weight, 2975 grm. 2 c.c. injected; animal killed two and a half hours later. 42 c.c. blood with 1 c.c. potassium oxalate; centrifugalised for three hours. Total amount recovered, 6·21 mg. chloroform. Corpuscles, 76·4 per cent.; plasma, 23·6 per cent. of total chloroform present.

Rabbit H.—Weight, 1450 grm. 1·5 c.c. injected and repeated in 15 minutes. 30 c.c. blood and 1 c.c. potassium oxalate; animal killed two hours later; centrifugalised three hours. Total amount recovered, 8·25 mg. chloroform. Corpuscle, 78 per cent.; plasma, 22 per cent. of total chloroform present.

Rabbit Q.—Weight, 2300 grm. 3 c.c. injected and rabbit killed three hours later. 40 c.c. blood and 5 c.c. potassium oxalate; centrifugalised three hours. Total amount recovered, 5·88 mg. chloroform. Corpuscles, 74·8 per cent.; plasma, 25·2 per cent. of total chloroform present.

Rabbit T.—Weight, 2200 grm. 3 c.c. injected. 40 c.c. blood taken with 5 c.c. oxalate. Total amount recovered, 9·38 mg. chloroform. Corpuscles, 79·9 per cent.; plasma, 20·1 per cent. of total chloroform present.

Rabbit Y.—Weight 2050 grm. 3 c.c. injected. Animal killed two hours later. 40 c.c. blood taken with 10 c.c. oxalate; centrifugalised three hours. Total amount recovered, 10·84 mg. chloroform. Corpuscles 72·4 per cent.; plasma, 27·6 per cent. of total chloroform present.

Average distribution in six rabbits:—Corpuscles, 77·2 per cent.; plasma, 22·8 per cent. of total chloroform present.

Consideration of Results.

Where the chloroform was inhaled the amount found in the plasma varied from 9·2 to 14·8 per cent. of the total amount recovered from the blood. Where the chloroform was injected the amount found in the plasma varied from 18·4 to 27·6 per cent. of the total amount recovered from the blood. Thus in all cases where the chloroform was

given subcutaneously the amount recovered from the plasma was much more than where the anæsthetic was inhaled. The delayed elimination therefore appears to be due to the different fixation of the drug in these cases.

The corpuscles appear to part with the chloroform rapidly, and after anæsthesia by inhalation the proportion held by the corpuscles is rapidly eliminated. Probably this is also the case with the proportion of chloroform held by the corpuscles after injection, but the difficulty of getting rid of the amount in the plasma seems to account for the delay in elimination and the injury to kidney and liver tissue.

These results suggest that in cases of delayed chloroform poisoning the distribution of the anæsthetic in the blood may more closely approximate to that of rabbits after injection of chloroform. The amount in the plasma varies within fairly wide limits, and this variation may be due to a variety of causes. Anything which would cause a larger proportion than usual of the chloroform in the blood to combine with the plasma constituents might cause a delayed elimination of the drug, and consequently injury to liver and kidney tissue.

It is of interest to note that in one rabbit which died just before the chloroform inhalation was stopped—after one hour inhalation—the amount of chloroform recovered was 22·24 mg. in 35 c.c. of blood, and of this the corpuscles contained 67·7 per cent. and the plasma 32·3 per cent., so that the fatal result was at least associated with a large percentage of chloroform in the plasma.

Summary.—Evidence is given that in rabbits the blood contains a larger proportion of chloroform in the plasma when the anæsthetic is given subcutaneously than when it is given as an inhalation. This is considered to be the reason for the delay in elimination and the consequent greater injury to the tissues which is associated with this mode of administration.

A grant was received from the Carnegie Trust to defray the expenses of this research.

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The Effects of Chloroform.

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(*Reprinted from the "Glasgow Medical Journal," July, 1912.*)

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THE EFFECTS OF CHLOROFORM.[1]

BY G. HERBERT CLARK, M.B., D.P.H.,
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THE choice of a subject for this paper which I am giving you this evening was in some respects a difficult matter, and it may appear to you that the title, "Effects of Chloroform," suggests something much more closely allied to surgery than physiology. This is not actually the case, for, although the subject is not of physiological interest alone, much of the best work on the subject has been done by physiologists. In the immediate and remote effects, which I wish to refer to, I have no intention of entering into details as to the condition of the pupil, &c., such as are ordinarily taught in classes of clinical surgery; but wish to indicate to you more particularly the effects of the drug upon the tissues, and upon metabolism and excretion. In many respects a more complete knowledge of these effects is necessary in practical medicine and surgery than is popularly taught, and it is with the purpose of introducing to your notice some of the less well known older work on the subject and the recent work, part of which has been done in the physiological department here, that I venture to come before you.

In considering the effects of chloroform upon any animal

[1] A lecture delivered to Queen Margaret College Medical Club on 15th January, 1912.

there are a number of points to which we must direct our attention. Foremost of these is the question, "How does the drug reach the tissues?"

In the great majority of cases chloroform is administered in the form of an inhalation—the vapour of the anæsthetic being inspired along with air. Thus the chloroform-air mixture passes to the lungs, and in due course a dilute mixture comes in contact with the rich plexus of blood-vessels coursing along the alveolar walls. Here the oxygen enters into a loose chemical relationship with the hæmoglobin, and is thus carried to the tissues. The fate of the chloroform is not so clearly defined, for, whereas it seems proved beyond question that the larger part of what is absorbed is carried by the red corpuscles, the plasma contains, according to Nicloux,[1] more than 12 per cent of the total chloroform in the blood.

Then there is the question, "What particular element in the corpuscles and plasma does the chloroform form loose compounds with?" for the compounds must be loose if the blood is to pass the chloroform on to the tissues. Here, again, we have some difference of opinion. Some (Moore and Roaf)[2, 3] hold that the proteins form unstable compounds with the chloroform. They argue that "since proteins build up the living protoplasm, chloroform and other anæsthetics must form similar unstable compounds with protoplasm, and that anæsthesia is due to the formation of such compounds which limit the chemical activities of the protoplasm. On account of the unstability of the compounds, these remain formed only so long as the pressure of the anæsthetic in the solution is maintained." Others[5, 6] hold that the "lipoid" element in the corpuscles, plasma, and tissues is responsible for the fixing of the chloroform, so long as the vapour pressure of the chloroform is sufficient. The term "lipoid" is held to mean those "fat-like" constituents of animal or vegetable cells which can be extracted by means of ether or similar solvents" (Rosenheim[7]). Meyer[6] has postulated the hypothesis that all bodies capable of dissolving fats possess, in a greater or less degree, anæsthetic properties. This suggestion as to the importance of the "lipoids" in anæsthesia is supported by the experiments of Nicloux,[8] who showed that, if an animal was anæsthetised with chloroform and killed when fully anæsthetised, the nervous tissue of the brain and spinal cord contained a larger proportion of chloroform than any other tissues—the blood excepted.

In further examining this point, Nicloux and Mdlle Frisson[9]

found that the white matter held more of the anæsthetic than the grey, and that the different power of fixation of chloroform varied with the proportion of fats or analogous substances. On pursuing this investigation with regard to other tissues they found that the law held good. Thus we may take it that the "lipoid" element in blood and tissue cell forms a loose combination with the chloroform, and that anæsthesia depends upon this.

It is also notable that the heart muscle is found to have a much greater affinity for chloroform than skeletal muscle, as represented by the figures 41 mg. per cent for cardiac and 21 mg. per cent for skeletal muscle. Sherrington and Sowton [10] have shown that striped muscles—unlike cardiac—are not readily poisoned by chloroform. Thus the substances which fix the chloroform in cardiac muscle are present in smaller amount in skeletal muscle, and we have a much smaller susceptibility of the latter to the action of the drug.

Having thus decided that the chloroform owes its action to its affinity for "lipoid" substances, we must now consider how the anæsthetic is absorbed. Is it taken up rapidly or slowly? Is it taken up at a uniform rate?

The work of Brodie and Widdows [11] and Buckmaster and Gardner [12] agrees in all important respects in this connection. They show that the chloroform absorption increases with great rapidity in the initial stages of anæsthesia to a value which approaches a maximum.

Brodie and Widdows show that the period of maximum absorption is during the second minute of the administration, but Buckmaster and Gardner place the time somewhat later, from five to fifteen minutes in three instances.

Following this maximum of absorption the breathing becomes shallower, probably owing to the effect of the chloroform on the respiratory centres. This depression of respiration varies in different individuals, and appears to depend on the degree of concentration of the chloroform-air mixture. During the period of respiratory depression the rate of absorption diminishes.

At this stage in animals the authors found that there was danger of the respiration completely stopping, and that, although artificial respiration was frequently successful in restoring the breathing, this was not always the case. Buckmaster and Gardner state that all the deaths they had during this period were entirely due to respiratory failure, and none to cardiac failure. Levy,[13] on the other hand, has recently published a communication in which he records

18 cases of sudden death in cats during light anæsthesia, in which fibrillation of the ventricle was the cause of death. He points out that these deaths occurred when the animals were imperfectly anæsthetised, and that the removal of and re-application of the inhaler appeared to play a part in the fatal result.

In any case, whichever is affected most markedly, the evidence is completely in agreement that a definite danger-point occurs within the first few minutes of anæsthesia. This is represented by the period between Y and Z in Fig. 1.

Sherrington and Miss Sowton have shown that cardiac muscle is much more readily affected by chloroform solution plus CO_2 than by chloroform solution plus O_2, and, consequently, the period of diminished ventilation of the lung must necessarily be one of danger.

If the animal passes this stage, and the administration is continued, the absorption again increases, and the chloroform content of the blood rises again until a point of equilibrium is reached—intake and output going on side by side. This equilibrium can be maintained for a considerable time, which varies in different individuals. The point of equilibrium is, however, not one of safety, for the difference between the amount of chloroform in the blood throughout this stage and death is very small.

At the same time, as absorption of the anæsthetic is going on, elimination is also taking place, and, as in the case of absorption, this does not take place at a uniform rate.

The rate of elimination to some extent depends upon the condition of the animal under experiment, but generally it may be taken that the rate of output is rapid at first and slower after. When the point of equilibrium referred to above is reached, a very small administration of the drug is all that is necessary to keep the animal anæsthetised—that is to say, both absorption and elimination are slow. The initial rate of elimination is slower than the initial rate of assumption, so that, as pointed out before, the blood is all the time becoming more charged with chloroform.

After the administration is stopped the rate of elimination is at first very rapid; indeed, according to Nicloux,[14] half the chloroform content is eliminated in the first five minutes in dogs. Buckmaster and Gardner[15] found that half the chloroform content was eliminated in fifteen to twenty minutes in cats, and in rabbits Paton and Miss Lindsay[16] found that in dogs more than half the chloroform was eliminated in twenty-five minutes.

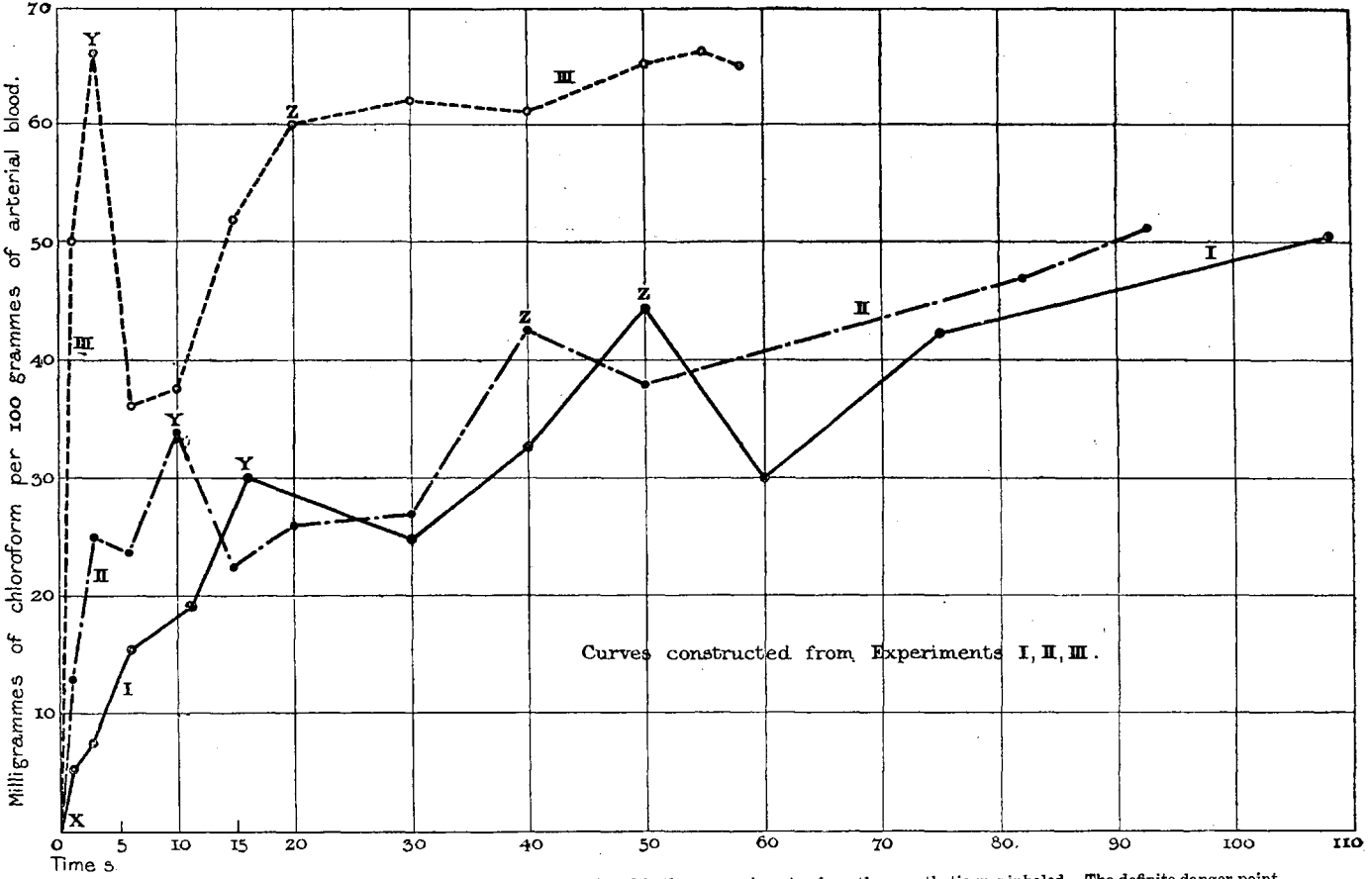

FIG. 1.—The curves show the rapid assumption of chloroform by the blood in three experiments where the anæsthetic was inhaled. The definite danger-point exists between Y and Z, when ventilation and consequently assumption of chloroform is reduced (Buckmaster and Gardner).

The remaining part was eliminated at varying rates. In some instances very little sign of chloroform was found after two hours, although in others about 20 mg. per cent was found—an amount equal to about one-half of what was required to produce full anæsthesia. It is of interest to note that in these cases the animals were kept in small cages and at rest.

After more than three hours a relatively large amount may

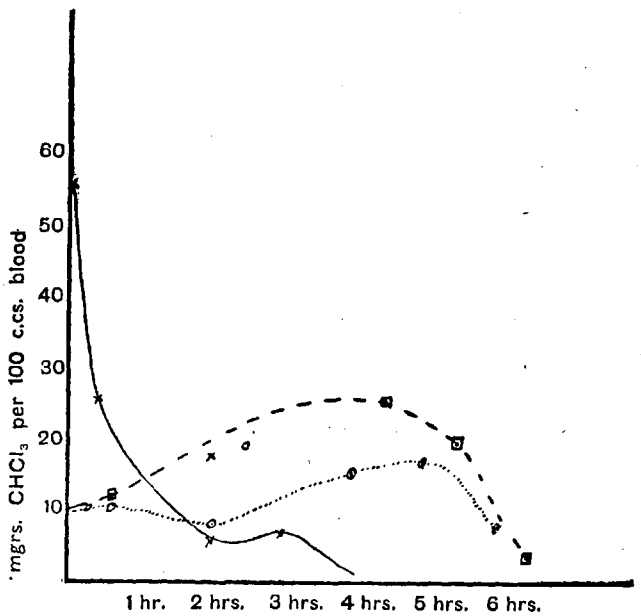

FIG. 2.

Curves showing the rate of assumption and elimination of chloroform when given in the three ways indicated (Paton and Miss Lindsay).

——— By inhalation.
. . . . . By injection.
— — — — — By stomach.

still remain. Such instances may probably be considered abnormal, and we may take it that normally the elimination of chloroform, when given through the respiratory tract, is comparatively rapid, particularly if the administration has been of short duration.

When chloroform is given in ways other than through the

respiratory tract, the rates, both of absorption and elimination, are very different.

It is well to look into these points, for it is quite a common thing for chloroform in one form or another to be given by the stomach, as flavouring agent in a mixture, or for its soothing effect in cough, &c., and Giani[17] has recently published a paper advocating its use as an anæsthetic in the form of intravenous injection.

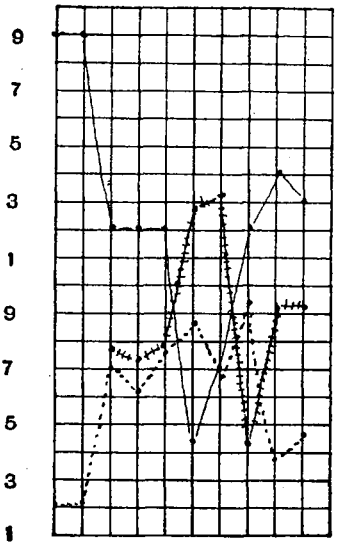

FIG. 3.

Curves showing the distribution of the urinary nitrogen after chloroform administered by the stomach (Paton).

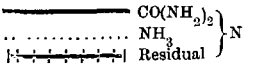

Fig. 2 shows the much slower rate of assumption of chloroform when given by the mouth or hypodermically, and also indicates the very much greater length of time required before elimination is anything like complete.

The maximal amount in the blood is not reached until about four hours after administration by the stomach, and not until about five hours after injection.

Thus when administered in either of these latter ways the

tissues are affected by chloroform for a very much longer time than when the drug is given in the more ordinary way. This point is of very great importance, as we shall see when we examine the effects upon metabolism and upon the tissues themselves.

When chloroform is administered through the respiratory passages the effect upon metabolism is at best but slight. These effects, if present, would be indicated, in degree at least, by changes in the excretion of the products of disintegration in the urine.

Quantitative change in the protein broken up would be shown by alterations in the amount of nitrogen excreted, while quantitative changes would be indicated by alterations in the distribution of the excreted nitrogen and sulphur in the constituents of the urine containing these elements. In experiments performed in dogs by Noël Paton[18] any effect that followed the administration by inhalation was of the nature of a stimulation of the hepatic metabolism. This showed itself in an increased conversion of ammonia to urea. Thus the proportion of N as urea remained unchanged or slightly increased, while the Nitrogen in NH_3 was unchanged or slightly decreased.

When given by the stomach the protein disintegration caused by the administration of chloroform is very greatly increased, particularly on the day of or the day after the administration. After a day or two the proportions of the various N, containing substances in the urine, undergo marked changes, the urea N diminishes, the NH_3 N increases, and later the residual N increases (Fig. 3). The increase in the latter is quite marked, and Paton suggests that it probably represents the appearance of amino acids. The proportion of unoxidised S to total S shows a distinct fall.

With the idea of throwing some light upon the nature of the products forming the residual N, and thus testing Paton's hypothesis as regards the presence of amino acids, Miss Lindsay[19] has recently published a series of complete analyses. Experimenting on three dogs she found, in addition to the increase in total nitrogen and the diminution in urea nitrogen, with rise in NH_3 nitrogen, that the residual N could be divided into allantoin N, amino acid N, creatinin N, and creatin N with a small percentage of undetermined nitrogen.

A slight rise in allantoin N is noted, which is probably caused by the toxic action of the drug upon the liver cells.

The amino acid N rose in each case, the rise generally

being in mon-amino acids, but in one case the rise was probably due to increase in di-amino acids and polypeptides. The creatinin N remained practically unchanged, but creatin appeared in the urine two or three days after the administrations. This disturbance of the creatin metabolism is associated with the disturbance in the hepatic functions, and Underhill and Kleiner [20] found fatty degeneration and creatin excretion following injection of hydrogen sulphate, while Mellanby [21] found creatin in considerable amount in cases of carcinoma of the liver. The amount of creatin in the urine may be taken, according to Hoogenheryze and Verploegh,[22] as an indication of the extent of the hepatic disturbance.

The undetermined nitrogen probably represents di-amino acids and polypeptides.

These effects upon the metabolism suggest that when given by the mouth chloroform exerts a toxic action upon the liver cells, notably, a day or two after the administration. The urine also shows the presence of tube-casts, renal epithelium and protein, so that the kidney is affected also. This is much less marked when chloroform is given as an inhalation.

When chloroform is given hypodermically it acts in the same way as when given by the mouth, but not so markedly. The toxic effect upon the liver cells does not appear to be as great as when the drug is given by the mouth. There may be a fall in urine N, with a rise in residual N and in ammonia N, but in other instances there may be no definite change in the nitrogen excretion.

Thus it would seem as if the rapid assumption of chloroform, when administered through the lungs, and the relatively rapid elimination, stimulate the hepatic metabolism; whereas the chloroform, more slowly absorbed and eliminated, and therefore having a much more prolonged action when given by the mouth or hypodermically, leads to an actual injury of the liver and kidney cells.

Another element may influence the rapidity of assumption and elimination of the drug. Moore and Roaf (*loc. cit.*) have shown that chloroform is relatively very soluble in blood plasma, and it appears possible that the solubility of chloroform in plasma plays a part in its elimination. For example, the chloroform, combined with the lipoids of the corpuscles after inhalation, appears to be loosely combined, and, consequently, the chloroform is fairly rapidly eliminated, more than 50 per cent within half an hour of the cessation of the administration.

When given by stomach or hypodermically the assumption of the drug is slow, and as the drug is absorbed into the blood-stream itself it seems probable that a quantity of it will remain dissolved or fixed in the plasma, and, consequently, its destructive action upon liver and kidney cells will be much more marked.

During anæsthesia from inhaling chloroform, at least 12 per cent of the chloroform in the blood is found in the plasma, and it may possibly be this small percentage which, in some animals referred to by Noël Paton and Miss Lindsay, appeared in the blood three or more hours after the withdrawal of the anæsthetic.*

The influences of chloroform upon metabolism having been thus briefly referred to, I wish to direct your attention to the effects of the drug upon the various tissues, as these explain in great part the influence upon metabolism.

In the first place the chloroform is absorbed into the blood-stream, and we have its stimulating or its toxic action first upon the blood-cells. *In vitro* chloroform is found to cause laking of the blood, owing to the solution of the lipoids of the red cells. This, however, is with strong solutions of chloroform. When administered in the three ways described before, I have found experimentally that a rise in the number of red corpuscles per c.mm. follows next day, and persists for some days.

There does not appear to be a similar increase in leucocytes, and Dawson,[23] in two dogs, found a slight fall. This may, in measure, be explained by the exudation of leucocytes into the renal tubules, as referred to by Thompson.[24]

Last year I made a large number of experiments upon rabbits with the object of finding if the increase in red corpuscles was due to concentration of the blood, but arrived at no definite result. The rise in red corpuscles in the rabbit is large, amounting at times to as much as 25 per cent, counting by the Thoma-Zeiss hæmocytometer, and occurred almost invariably after administration. In the small number of cases which did not so react most of the animals died very shortly after. The increase after injection or administration by the mouth was greater than when the drug was inhaled. The increase is probably due to the large loss of water in the urine following the administration, as observed by

* Since delivering this lecture, the author and Miss Lindsay have definitely determined that the proportion of chloroform in the plasma is very much greater when the drug is given hypodermically than when inhaled.

Thompson (*loc. cit.*). Microscopically the red cells showed signs of degeneration in some instances, and the varied size of the erythrocytes and the activity of the bone-marrow suggest

TABLE I.*—SHOWING AVERAGE NUMBER IN MILLIONS OF CORPUSCLES PER C.MM. AFTER CHLOROFORM GIVEN IN THE WAY INDICATED.

	Inhalation.	By Stomach.	Injection.
Day of administration, . .	6·3	5·8	5·9
1st day after, ...	7·2	7·3	7·4
2nd day after, ...	7·1	6·9	6·8
3rd day after, ...	6·7	6·8	6·5
4th day after, ...	6·1	6·9	6·4

that the blood-forming tissue was actively forming cells to make up for those injured by the administration.

The table (I) shows the average rise and fall after administration in the three different ways. In the inhalation

TABLE II.*—ADMINISTRATION BY INHALATION.

No.	Day.									
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.
6	1470	1450	—	—	—	—	—	—	—	—
7	950	920	860	770	—	—	—	—	—	—
8	2250	2250	2120	—	—	—	—	—	—	—
12	1850	1725	1750	1780	1880	1855	1850	1870	1855	1750
20	2300	2275	2270	2270	2250	2200	2400	—	—	—
22	2050	1975	1950	1900	1850	1850	1800	1750	1700	1625*
24	2250	2170	2250	2150	2250	2250	2100	2150	—	—
26	1600	1600	1700	1750	1750	1750	1700	—	—	—
27	1650	1600	1550	1700	1750	—	—	—	—	—
30	2050	1950	1800	1670	1600	—	—	—	—	—
32	2370	2270	2260	2250	2300	2100	2050	1950	1925	1950†
36	1600	1550	1550	1550	1600	1600	—	—	—	—

* Survived for one month. Weight at death 1400 grammes.
† Survived for two months. Weight at death 1840 grammes.

experiments chloroform was exhibited for periods varying from fifteen minutes to one hour. Given by the stomach, or injected hypodermically, 1 c.c. was administered.

* These tables are reprinted by kind permission of the editor of *The Lancet.*

In addition to these effects upon the blood we have effects upon the liver, kidney, spleen, and heart which have to be considered histologically; but first I wish to draw attention to the effects of chloroform upon urinary secretion at the time of administration and immediately after. According to Thompson, who conducted a series of experiments on dogs, which were anæsthetised by inhalation for periods of from two to four hours, the quantity of urine secreted increases during the early stages when the anæsthesia is light, but is diminished or suppressed during full anæsthesia.

During chloroform anæsthesia the urine secreted is invariably more dilute than normal, even with diminished amount. He finds that there is a general but not accurate correspondence between urine outflow, kidney volume, and blood pressure. After the removal of the anæsthetic the amount of urine secreted increases greatly, sometimes as much as four times the normal. The maximum outflow occurs about three hours after removal of the anæsthetic. Thompson reports also considerable exudation of leucocytes into the renal tubules, particularly after prolonged necrosis.

Albumen and reducing substances, other than glucose, in increased amounts are frequently found, and the excretion of chlorides is much increased, both during and after anæsthesia.

This summarises the effect of chloroform upon kidney excretion, and we have to turn to histological investigation to find causes for the metabolic and excretory disturbances which have been referred to. In experiments performed by the author[25] the effects upon the various tissues varied considerably with the manner of administration and with the amount given. When administered by inhalation for a short period a comparatively slight change was all that was seen, except in rare instances. A small degree of cloudy swelling was frequently seen in kidney and liver cells, but this was absent a day after the administration.

When chloroform was administered to rabbits by the stomach the mortality was very great, and in those animals which survived extensive changes in the liver and kidneys were invariably found soon after two hours.

The kidney tissue showed marked degenerative changes. The tubules were frequently found to be choked with albuminous *débris*. The cells were broken down, and the nuclei stained unequally, some taking the basic stain very badly. Granules giving a positive reaction for fat were found in large quantities. Signs of hæmorrhage were frequently

found, and in the cells lining the tubules adjacent to the hæmorrhages dark granules of hæmosiderin were found.

Still greater changes were found in the liver.

In the animals which suffered most the centre of the liver lobule was found to be broken down into a cheesy mass showing no signs of liver tissue, and containing large masses of *débris* giving a positive reaction for fat. The periphery of these lobules invariably showed cells closely resembling normal liver cells, while the intermediate zone showed cells in various stages of degeneration.

In animals that were somewhat less affected by the drug, or which were killed soon after the administration, the cells in the centre of the lobules were found to be markedly degenerated, while the intermediate zone cells were less so, and the peripheral cells, to all appearance, very slightly injured.

When administered subcutaneously the changes caused by the chloroform are intermediate between those slight ones seen after inhalation and the extensive degeneration seen after administration by the stomach.

Chloroform in doses such as were given in the series of experiments caused enormous degenerative changes in the kidney and liver cells when administered by stomach, and somewhat less so when injected. This accords with the results obtained by Doyon.[26] These facts explain the metabolic changes already observed. The slight changes seen after inhalation of the drug for a short time are in keeping with the slight metabolic changes referred to before. As to the probable reason for these different effects when chloroform is inhaled, the proportion of chloroform in the blood rises rapidly, and falls fairly rapidly. Assumption and elimination are both rapid, and the tissues are affected for a comparatively short time, except during a prolonged administration. When given by the stomach or hypodermically, assumption is slow and elimination is slow; and, consequently, the tissues are affected by the chloroform for a considerable time. This does not entirely explain the difference, for after a single prolonged administration by inhalation—say, for four hours—the amount of degenerative change in the organs is never comparable with that seen after, say, administration of 1 c.c. by mouth in both cases; the drug is almost completely eliminated after six hours. Sherrington and Miss Sowton (*loc. cit.*) have shown that when cardiac muscle is immersed in a solution of chloroform in saline a very much greater effect is produced than when

a similar quantity is dissolved in blood and serum. In the serum the chloroform is, in part at least, in combination with the proteins, whereas in the saline solution the chloroform is free and readily affects the muscle. The effect, therefore, depends upon the relative affinity of chloroform for saline, serum, and cell substance.

In whatever way the chloroform combines in the blood, it seems as if it may be fixed firmly or loosely, and that the anæsthetic effect is obtained by the loosely combined portion; while the destructive effects, seen after administration by mouth or hypodermically, are caused by a more firmly

TABLE III.—ADMINISTRATION BY SUBCUTANEOUS INJECTION.

No.	Day.											
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.
1	*1150*	*1150*	1100	1050	1070	*1075*	*1100*	1050	1070	1000	—	—
2	*2000*	1950	1890	*1925*	*1900*	—	—	—	—	—	—	—
3	*1550*	1550	1520	*1470*	*1550*	1450	1430	1450	—	—	—	—
	Daily Injections of 0·1 cc.											
4	1700	1680	—	—	—	—	—	—	—	—	—	—
5	1430	1400	—	—	—	—	—	—	—	—	—	—
13	2400	2450	2430	2230	—	—	—	—	—	—	—	—
16	2700	2630	2520	2625	2500	2470	2400	2310	2220	2180	2150	2130
19	1700	1680	1700	—	—	—	—	—	—	—	—	—
21	3100	3050	3050	3000	3050	3020	2975	2930	2900	2850	1 month	2725
25	1650	1550	1470	1450	1450	1350	1300	1350	1300	1375	1350	1250
28	1500	1400	—	—	—	—	—	—	—	—	—	—
29	1150	1150	1150	1150	1100	1060	—	—	—	—	—	—
31	2450	2420	2280	2250	2350	2450	2300	2150	2100	1950	26 days	1350
37	1325	1300	1200	1100	1200	1200	1150	—	—	—	—	—

combined portion whose elimination is delayed. It is instructive to note that in an animal, which was injected with 1 c.c. of chloroform and killed after five and a half hours, 31·6 mg. of chloroform per cent were found in the blood; while the animal, though shaky, was not completely anæsthetised, whereas full anæsthesia was produced by inhalation in a rabbit whose blood contained 30·8 mg. per cent when killed immediately after the administration. Another rabbit, which had 1 c.c. administered by the stomach, and which was also not fully anæsthetised, showed 32·5 mg. per cent in the blood when killed three hours after.

These observations in the delayed elimination of chloroform

throw some light upon the condition known as late chloroform poisoning, as the lesions found in liver particularly, and in part in the kidney, are similar to those described by various authors as occurring in late chloroform poisoning.[27, 28, 29, 30, 31]

TABLE IV.—ADMINISTRATION BY THE STOMACH.

No.	Day.											
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.
9	2380	2320	2200	2250	2250	2160	2210	2175	—	—	—	—
14	2300	2350	2380	2380	2240	—	—	—	—	—	—	—
15	2150	2025	1975	2050	2150	1950	1925	1900	1940	1875	1870	1870
18	2400	2520	2500	2550	2450	2300	2150	4 months later 1350				
23	1700	1700	1750	1700	1650	1550	1500	1500	1450	—	1 month	1620
33	2100	2000	2000	1900	1900	1800	1700	1650	1600	1550	1475	—

The observation made by Noël Paton and Miss Lindsay, that the rate of elimination is improved by free ventilation and exercise in rabbits, suggests a method of minimising risk of this sequel to chloroform anæsthesia, and emphasises the importance of not letting a patient "sleep it off." The condition seems to depend upon delayed elimination, and

TABLE V.—WEIGHT OF THE SPLEEN IN ANIMALS ANÆSTHETISED.

No.	Original weight of Animal.	No. of days.	Weight of spleen.
12	1850 grammes.	20	2 grammes.
20	2300 ,,	7	0·9 gramme.
22	2050 ,,	31	1·2 grammes.
24	2250 ,,	8	1·95 ,,
26	1600 ,,	7	0·6 gramme.
27	1650 ,,	5	1·4 grammes.
30	2050 ,,	5	0·72 gramme.
32	2370 ,,	65	1·2 grammes.
36	1600 ,,	6	1·2 ,,

this may be due to the fixation of a certain proportion of the chloroform in the proteins of the serum or tissues.

So far I have considered only the single administration of relatively large doses, 1 c.c. injected or by stomach. The results in these experiments quoted were very striking,

and suggest that a very much smaller dose might have been sufficient to cause changes in the tissues, particularly if given frequently. The great interest in this particular series of observations lies in the fact that while 1 c.c. of pure chloroform is probably never given to human beings

TABLE VI.—THE WEIGHTS OF THE SPLEENS AND OTHER PARTICULARS.

No.	Original weight of Animal.	No. of days.	Weight of spleen.
16	2700 grammes.	13	1·5 grammes.
21	3100 ,,	30	1·85 ,,
25	1650 ,,	15	0·67 gramme.
28	1500 ,,	3	1·2 grammes.
31	2450 ,,	26	1·5 ,,
37	1325 ,,	7	1·5 ,,
Average,	2121 ,,	16	1·37 grammes.

by the mouth, the small dose given to rabbits in this way corresponds to the amount of chloroform found in a single licquorice, linseed, and chlorodyne lozenge, (Crichton Browne [32]).

Enough chloroform water to contain 0·08 gm. of chloroform

TABLE VII.—THE WEIGHTS OF THE SPLEENS AND OTHER PARTICULARS.

No.	Original weight of animal.	No. of days.	Weight of spleen.
14	2300 grammes.	5	1·2 grammes.
15	2150 ,,	14	0·3 gramme.
18	2400 ,,	140	1·5 grammes.
23	1700 ,,	30	0·95 gramme.
33	2100 ,,	11	0·8 ,,

was given each day to a number of rabbits, and observation made upon them (Clark) [33]. Similarly 0·1 c.c. was injected daily into another group of rabbits, while a third lot were anæthetised through the lungs for ten minutes on alternate days. In the latter case daily administration was found

to be very fatal, only the first three animals used having daily administrations.

Valuable information was obtained from a critical study of the weights from day to day.

1. *Inhalation.*—Where the administration took place daily the weight fell rapidly, but where the administration was on alternate days there was but little change, unless where the animal lived for some time (Table II).

2. *Injection.*—Here the effect is very marked, and the animals lost weight fairly rapidly, as is shown in Table III.

3. *By stomach.*—Here, also, the loss of weight is marked. One animal, however, survived for four months, but its weight diminished almost 50 per cent in that time (Table IV).

The histological changes seen were similar to those already described, but these were found in all cases, and not only in a proportion of them. While both kidney and liver suffered in all the animals used, the kidney suffered relatively more after inhalation than when chloroform was administered in the other ways.

The spleens were also examined, and showed in all cases great engorgement, and often very great increase in size and weight.

The average weight for four control rabbits weighing over 2,000 grms. each was 0·78 gm., and the tables show the enormous increase in the various different ways of administering chloroform (Tables V, VI, and VII).

Histologically the sinuses were engorged with blood, and a large amount of iron containing pigment (hæmosiderin) was seen frequently filling large phagocytes, which were seen in all the sections examined.

The hearts in many animals showed degenerative changes, particularly after the inhalation experiments. Loss of cross striping and segmentation of the fibres were features, and also imperfect staining of the nucleus.

Considering these results it seems apparent that given in the form of small doses, frequently repeated, chloroform is a much more dangerous drug than when given in a single much larger dose. The first doses given appear to lower the vitality of the tissues, so that the later doses have a more marked action.

In conclusion, the experiments cited seem to prove that chloroform, however given, has far-reaching effects on the tissues and blood, and, consequently, upon metabolism and

excretion. These effects are enormously increased when elimination is delayed.

Elimination may be delayed in many ways, and idiosyncrasy, or a number of undetermined factors, appears to influence the action of chloroform on animals.

A danger-point occurs soon after the commencement of the administration by inhalation, and, although the establishment of equilibrium between assumption and elimination marks a point of less danger, the safety is only relative. Immediately following the administration changes of a degenerative nature may appear in many organs, and with delayed elimination the occurrence of delayed chloroform poisoning in some degree is certain to occur. Thus it seems proved that chloroform, however given, is an exceedingly treacherous drug, and that its use is fraught with grave danger.

A grant was received from the Carnegie Trust to defray the expenses of this research.

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THE INFLUENCE OF CARBON DIOXIDE ON THE HEART IN VARYING DEGREES OF ANÆSTHESIA.

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Reprinted from the Journal of Physiology.
Vol. XLVII. Nos. 4 & 5, December 19, 1913.

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THE INFLUENCE OF CARBON DIOXIDE ON THE HEART IN VARYING DEGREES OF ANÆSTHESIA.

By E. P. CATHCART and G. H. CLARK.

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In the course of a research carried out by one of us (E. P. C.(1)) on the influence of work upon the gaseous metabolism the subject suddenly collapsed, during two experiments, whilst doing hard work and whilst in the circuit of the respiration apparatus. In each case it was noted that the collapse was preceded by a sudden and rapid rise in the pulse-rate. It was thought that possibly this alteration in the cardiac condition was produced as the result of carbon dioxide poisoning, *i.e.* owing to the defective absorption by the soda lime there was an accumulation of carbon dioxide in the circuit. In order to investigate further the question of this possible influence of carbon dioxide on the heart the present experiments were started. It was soon found however that the conditions of the work experiment were very difficult to repeat and that it was practically impossible to investigate the influence of the carbon dioxide on the heart of the normal unanæsthetised animal. Experiments were therefore begun with the animals under the influence of ether. For a long time we were quite unable to obtain consistent results, and at first we ascribed the variations which we obtained to individual idiosyncrasies on the part of the animals (rabbits) used, as Mares(2) had previously found that the rabbit is one of the most insensitive of animals to the action of carbon dioxide. He maintains that some rabbits can inhale a gaseous mixture consisting of 80 % CO_2 and 20 % O_2 without trouble of any kind for some twenty minutes. We discovered in the end however that the differences were due to a readily controllable cause, namely the depth of the anæsthesia, during the course of the administration of the carbon dioxide. The present communication deals with the influence of the depth of anæsthesia on the effect of carbon dioxide on the heart and blood-pressure.

Previous work. So far as we are aware there is no previous experimental work on the effect of carbon dioxide on the heart of intact animals in varying degrees of ether anæsthesia. The only paper which deals with this particular point is a very interesting report by Prof. Sherrington and Miss Sowton (3) on the effect of chloroform in conjunction with carbon dioxide on cardiac and other muscle. These workers found that, in the case of the perfused heart, the cardiac muscle was distinctly more sensitive to chloroform when this anæsthetic was given in unoxygenated saline containing carbon dioxide instead of in oxygenated saline. A further paper by Goodman Levy (4) on the variation in cardiac "irritability" during varying degrees of anæsthesia, is of considerable interest in this connection. He found that under the influence of chloroform the mammalian heart is in an irritable condition and that this irritability is raised under conditions of light anæsthesia.

As regards the effect of carbon dioxide alone upon the heart there has been a considerable amount of work done. Öhrwall (5) found that carbon dioxide in large amount exercised a paralysing effect on the cardiac musculature (isolated frog heart) but that this paralysis could be caused to disappear by the subsequent free use of oxygen. Mares (2) also showed that the effect of the administration of carbon dioxide was quite characteristic. The systole became progressively weaker so that in the end the heart passed into a condition of fibrillation. He believed as did also Benedicenti and Treves (6) that this effect was due to the direct action of the carbon dioxide on the cardiac muscle. Gross (7) found that carbon dioxide saturated Ringer solution perfused through the isolated mammalian heart brought about a slowing and weakening of the cardiac activity which might even lead to complete cessation of the heart's action. The same effect has also been observed in the hearts of such simple forms as *Aplysia limacina* by Straub (8) and of *Helix pomatia* by Lovatt Evans (9).

Recently Starling has taken up the consideration of this subject. He had previously, working in conjunction with Kaya (10), found that when he used an intact animal, an increased carbon dioxide tension in the blood caused a rise in blood-pressure and that on the cessation of the carbon dioxide inhalation, the blood-pressure fell slowly to its original level. If however a spinal animal was used the administration of carbon dioxide in the presence of adequate amounts of oxygen did not produce any result on the blood-pressure although in some instances they noted a slight increase in the amplitude of the heart beat. This work was continued by Starling in conjunction with Jerusalem (11),

In their experiments an isolated heart lung preparation was used. In the case of the cat's heart they found that if the heart were fed with a current of blood under constant normal arterial pressure—the artificial ventilation with varying amounts of carbon dioxide being done by the lungs—12–20 % of an atmosphere of carbon dioxide produced cardiac dilatation, the systolic volume being more affected than the diastolic volume with as a result a diminution in the ventricular output, whereas with 2–8 % of an atmosphere of carbon dioxide the ventricular output increased. They further found that carbon dioxide caused slowing of the isolated heart, a condition which became increasingly marked with increase in the carbon dioxide tension. The increased ventricular output they held must be a contributory factor in the general rise of blood-pressure which resulted from the inhalation of carbon dioxide as observed in the previous experiments carried out by Kaya.

Mathison (12) continued the observations on the effect of carbon dioxide on the spinal animal. He found that, when he employed larger percentages of carbon dioxide than were used by Kaya and Starling, the effect on the blood-pressure was very variable, sometimes it was but little changed and at other times there was a marked rise in the blood-pressure. As regards the heart volume he found, in opposition to Jerusalem and Starling, that with percentages of carbon dioxide up to 40 and 50 % the systolic output of the heart increased. This difference he believed was due to the limitation of the circulation in the isolated heart lung preparation. On the removal of the carbon dioxide the heart rapidly returned to normal. Vaso-constriction probably plays a part in the changes of blood-pressure.

Itami (13), working under the direction of Starling, attempted to elucidate the factors which brought about the rise in blood-pressure following an increased tension of carbon dioxide in the blood. "In every case the animal was fully anæsthetised throughout the experiment with A.C.E. mixture, chloral hydrate or urethane, curare being in nearly all cases given in addition." The animals employed were usually small dogs although cats and rabbits were occasionally used. He found that with 5 % carbon dioxide the cardiac output increased and the blood-pressure rose slightly. With 8–10 % the cardiac output increased and a considerable rise in blood-pressure took place. With 12 % the heart dilated so that an actual decrease in output occurred, at the same time however the blood-pressure rose considerably. The cardiac output increased again if the administration of the carbon dioxide was persisted in. As the result of giving moderate percentages of carbon dioxide

there was usually slowing of the pulse. He thought that small percentages of carbon dioxide caused a rise in blood-pressure by direct action on the heart, whilst the percentages above 8% active constriction of the blood-vessels occurred from stimulation of the vaso motor centres.

Von Anrep's(14) experiments, also carried out under the direction of Starling, would tend to show that the alterations in blood-pressure, which were observed after the increase in the carbon dioxide intake, were due to an increased secretion of adrenalin acting on the heart as the rise did not take place after the extirpation of the suprarenal glands. In support of this contention of Starling is the recent work of Czubalski(15) who found that the rise in blood-pressure in asphyxia was due to an increased flow of adrenalin into the blood.

Method[1]. The method employed of obtaining the cardiac tracings was that already described by one of us (G. H. C.(16)). The rabbit was anæsthetised, a cannula inserted into the trachea and artificial respiration commenced. The heart was next exposed and the levers attached by means of small clips to the right auricular and ventricular walls. During the actual period of the experiments the artificial respiration was carried out by pumping with a hand bellows. The inlet of the hand bellows could be rapidly connected with a gas reservoir containing known dilutions of carbon dioxide and air (carbon dioxide and oxygen mixtures were sometimes employed with identical results). The carbon dioxide was obtained from a cylinder of compressed gas, and measured amounts of the mixture given. During the periods of hand pumping no further anæsthetic was given. In each case the hand pumping was started with ordinary air in order to get a comparative record and when the trace was steady the carbon dioxide mixture was administered. In every instance the preliminary hand pumping with air was carried out by the same observer who gave the carbon dioxide mixture so that the results might be uniform as regards rate and force. It was found with a little practice to be very easy to keep up the hand pumping steadily at the same rate and pressure for the few minutes the experiment lasted. The earliest tracings were made with two ordinary levers but the later ones were made by means of a very useful straight writing double recorder devised for us by the laboratory mechanic, J. McCall. In several of the later experiments the blood-pressure was recorded

[1] A demonstration of the method and of the influence of the carbon dioxide in varying depths of anæsthesia was given at the Internat. Congress of Med., 1913, Pharmacol. Section.

simultaneously with the cardiac contractions. During the intervals between experiments artificial respiration was carried out by means of a Brodie pump, the air passing to the animal through an anæsthetic bottle so arranged that the amount of ether given could be regulated at will.

A. *Heart experiments.*

I. *Influence of the anæsthetic alone.* The influence of the anæsthetic alone is a point which had to be considered and we carried out a number of observations on it. Our experiments have shown in common with the majority of others, carried out for this purpose, that, provided the administration of the ether is not pushed unduly, the anæsthetic has practically no effect on the rate and the amplitude of the cardiac beat. If the ether be given freely some inhibition of the amplitude takes place and if it be given in excess the heart goes into a state of fibrillation, or a condition closely resembling it, from which it recovers more or less readily if artificial respiration be carried out with air free from the anæsthetic. Vernon (17), who carried out a number of experiments on the isolated heart with the addition of ether to the perfusing fluid, found that the depressant action of the ether was proportional to the concentration.

II. *Influence of the inhalation of the carbon dioxide mixtures.* The effect of the administration of varying mixtures of carbon dioxide and air to an animal under the influence of ether varied according to the depth of the anæsthesia as is clearly demonstrated by the following protocols, tracings and Tables I and II which give a summary of a series of the experiments. In addition to the experiments summarised in these tables we have a very large number of experiments in which the results fall midway between deep and light anæsthesia as it is difficult to hit off always with certainty the proper depth of anæsthesia.

Protocol I (*a*). The rabbit was lightly anæsthetised and a mixture containing 16 % carbon dioxide in air was given. The amplitude of the excursion of both ventricular and auricular levers diminished markedly within ten seconds of the commencement of the experiment and the rate became slowed (see Fig. 1). After 30 seconds a slight struggle took place and the condition of the heart was so unsatisfactory that the experiment was stopped after three litres of the carbon dioxide air mixture had been given. The heart had been gradually failing and the marked dilatation of the auricles and the ventricles gave the trace a diastolic character. The measurements of the rate and amplitude are as follows:

	Commencement of experiment	Middle of experiment	End of experiment
Rate.			
Ventricle } in 3 seconds ...	8·5	7·0	5·0
Auricle } in 3 seconds ...	8·5	7·0	5·0
Amplitude.			
Ventricle } average of 3 contractions	29·0 mm.	26·7 mm.	11·3 mm.
Auricle } average of 3 contractions	8·3	7·3	5·7
Height of lowest point of ventricular trace above the base line	30·0 mm.	38·0 mm.	46·0 mm.

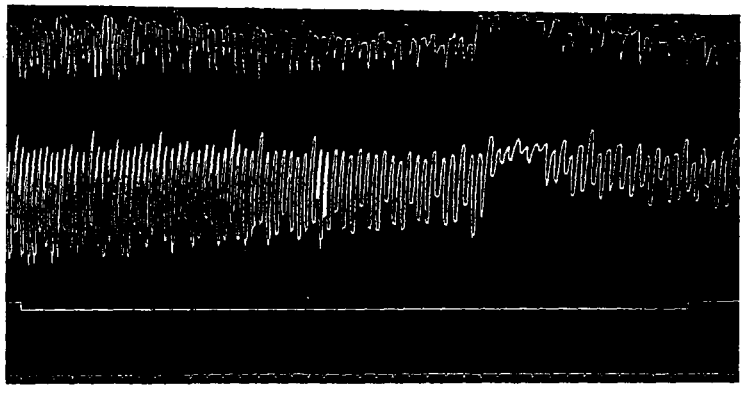

Fig. 1. Light anæsthesia. 3 litres of 16 % CO_2-air mixture.
[In this and all other traces the upper curves are auricular and the lower ventricular.]

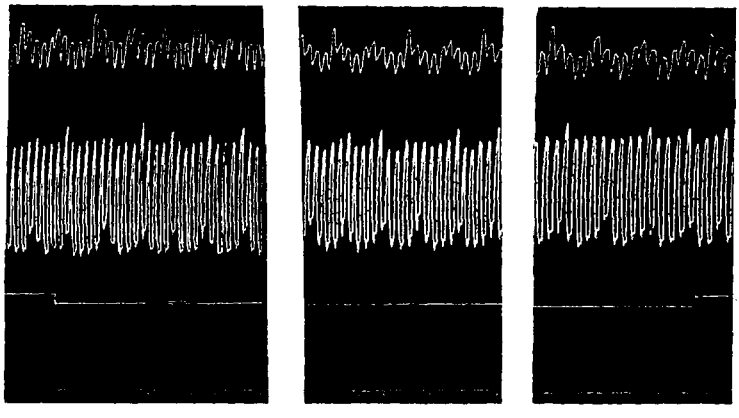

Fig. 2. Deep anæsthesia. 5 litres of 16 % CO_2-air mixture. Intervals=20 secs. Total time=72 secs.

(*b*) At the conclusion of Exp. (*a*) the ether was given until the animal was deeply under, no corneal reflex, the pupil widely dilated and inactive. Again 16 % carbon dioxide air mixture was given as before. A very slight diminution in rate took place but no change in the amplitude. Five litres of the mixture were given without markedly influencing the heart (see Fig. 2). The measurements of the rate and the amplitude are as follows:

	Commencement of experiment	Middle of experiment	End of experiment
Rate.			
Ventricle } in 3 seconds ...	9·0	8·5	8·0
Auricle } ...	9·0	8·5	8·0
Amplitude.			
Ventricle } Average of 3 contrac-	21·0 mm.	21·0 mm.	21·0 mm.
Auricle } tions	4·7	4·3	4·7
Height of lowest point of ventricular trace above the base line	22·0 mm.	23·0 mm.	24·0 mm.

Protocol II (*a*). A rabbit was lightly anæsthetised and five litres of a mixture of 12 % carbon dioxide in air were given. The amplitude of the auricular and ventricular contractions diminished slowly during the first 30 seconds after the administration began and then more rapidly, reaching a minimum 70 seconds from the commencement of the administration. The rate was slowed slightly at first but markedly during the second half of the administration (see Fig. 3). There was marked dilatation of the heart. Measurements were as follows:

	Commencement of experiment	Middle of experiment	End of experiment
Rate.			
Ventricle } in 3 seconds ...	11·0	10·0	6·0
Auricle } ...	11·0	10·0	6·0
Amplitude.			
Ventricle } average of 3 contrac-	85·0 mm.	51·0 mm.	37·0 mm.
Auricle } tions	4·0	—	—
Height of lowest point of ventricular trace above the base line	24·0 mm.	47·0 mm.	64·0 mm.

(*b*) At the close of the above experiment ether administration was pushed until the animal was deeply under. No corneal reflex, and pupils dilated and inactive. Five litres of 12 % carbon dioxide air mixture were given. A slight diminution in amplitude of the heart's contraction took place at first to be followed by a very small increase but no change in the rate (see Fig. 4). The measurements were:

	Commencement of experiment	Middle of experiment	End of experiment
Rate.			
Ventricle } in 3 seconds ...	8·0	8·0	8·0
Auricle } in 3 seconds ...	8·0	8·0	8·0
Amplitude.			
Ventricle } average of 3 contrac-	52·0 mm.	44·0 mm.	45·0 mm.
Auricle } tions ...	6·0	4·0	5·0
Height of lowest point of ventricular trace above the base line	55·0 mm.	62·0 mm.	63·0 mm.

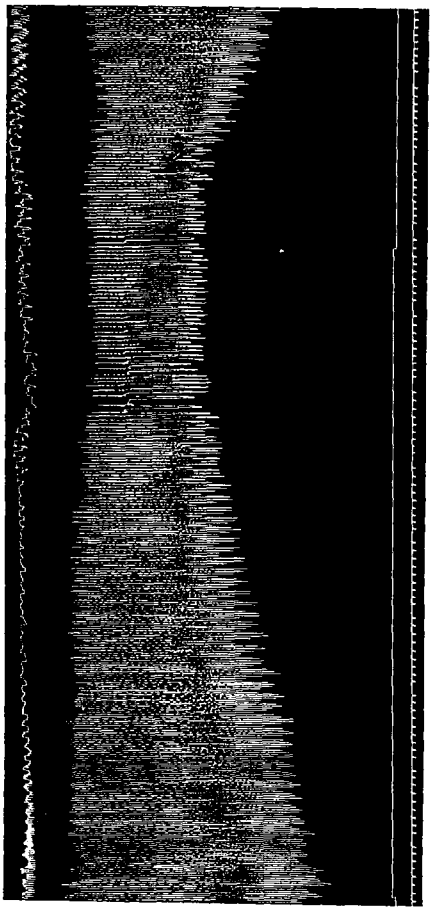

Fig. 3. Light anæsthesia. 5 litres of 12 % CO_2-air mixture.

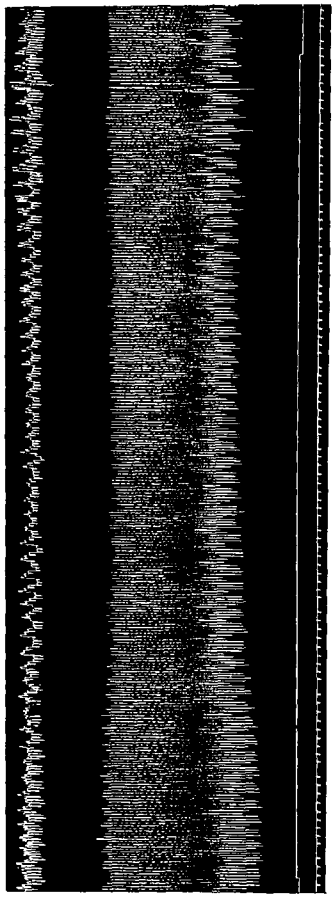

Fig. 4. Deep anæsthesia. 5 litres of 12 % CO_2-air mixture.

The results which we have obtained may be briefly summarised as follows. When the animal is lightly under the influence of ether (in all cases the animals were quite unconscious) the effect of the administration of carbon dioxide, by way of the respiratory tract, produces, with

TABLE I. *Light anæsthesia series.*

No. in series	Auricular & Ventricular *Rate*: Decrease 1st half	Decrease 2nd half	Increase 1st half	Increase 2nd half	Auricular and Ventricular *Amplitude*, in mm.: Decrease 1st half V	Decrease 1st half A	Decrease 2nd half V	Decrease 2nd half A	Increase 1st half V	Increase 1st half A	Increase 2nd half V	Increase 2nd half A	Total ventricular increase or decrease
1	2·5	0·0	—	—	5·0	1·0	10·0	—	—	—	—	0·8	−15·0
2	2·0	0·5	—	—	8·0	5·2	4·5	1·1	—	—	—	—	−12·5
3	0·0	3·0	—	—	2·6	—	3·4	7·0	—	0·6	—	—	− 6·0
4	3·25	2·75	—	—	19·0	6·4	—	10·0	—	—	0·6	—	−18·4
5	0·0	2·0	—	—	2·3	1·0	8·7	—	—	—	—	0·7	−11·0
6	2·0	2·0	—	—	9·0	—	—	—	—	—	1·7	—	− 7·3
7	1·5	2·0	—	—	2·3	1·1	15·4	1·6	—	—	—	—	−17·7
8	1·5	0·0	—	—	1·0	2·0	1·3	1·4	—	—	—	—	− 2·3
9	0·75	1·25	—	—	1·3	3·4	—	2·0	—	—	0·7	—	− 0·6
10	1·0	4·0	—	—	34·0	—	14·0	—	—	—	—	—	−48·0
	14·5	17·5	0·0	0·0	84·5	20·1	57·6	23·1	0·0	0·6	3·0	1·5	−138·8
Average for 10 Exps.:													
	1·45	1·75	0·0	0·0	8·45	2·01	5·76	2·31	0·0	0·06	0·3	0·15	−13·89

TABLE II. *Deep anæsthesia series.*

Degree of anæsthesia	No. in series	Auricular and Ventricular *Rate*: Decrease 1st half	Decrease 2nd half	Increase 1st half	Increase 2nd half	Auricular and Ventricular *Amplitude*, in mm.: Decrease 1st half V	Decrease 1st half A	Decrease 2nd half V	Decrease 2nd half A	Increase 1st half V	Increase 1st half A	Increase 2nd half V	Increase 2nd half A	Total ventricular increase or decrease
Deep ...	1	0·0	0·0	0·5	0·0	—	—	0·2	0·1	1·9	0·5	—	—	+1·7
Slight corneal reflex	2	1·5	0·0	0·0	0·0	4·1	0·6	2·0	—	—	—	—	2·0	−6·1
Deep ...	3	0·0	0·0	0·0	0·0	1·8	0·1	—	—	—	—	0·2	0·1	+1·6
Deep ...	4	0·5	0·0	0·0	0·0	1·0	—	—	—	—	—	—	—	−1·0
Deep ...	6	0·0	0·0	0·0	0·0	1·3	—	—	—	—	1·0	3·3	—	+2·0
Deep ...	7	0·0	0·0	0·0	0·0	0·2	—	—	—	—	—	1·4	2·2	+1·4
Slight C. R....	9	1·0	0·5	0·0	0·0	1·0	—	—	—	—	1·0	2·0	1·6	+1·0
Very deep ...	10	0·25	0·0	0·0	0·0	1·0	1·7	0·3	—	—	—	—	—	−1·3
Deep ...	11	0·5	0·5	0·0	0·0	—	0·4	—	—	—	—	—	0·4	0·0
C. R. after 20 secs.	12	0·25	1·25	0·0	0·0	0·3	—	0·3	—	—	0·6	—	1·0	−0·6
Slight C. R....	13	0·5	0·5	0·0	0·0	4·0	0·4	—	0·3	—	—	0·4	—	−3·6
Deep ...	14	0·0	0·0	0·0	0·0	8·0	2·0	—	—	—	—	1·0	1·0	−7·0
		4·6	2·75	0·5	0·0	22·7	5·2	2·8	0·4	1·9	3·12	8·3	8·3	−11·9
Av. for 12 Exps.		0·33	0·19	0·04	0·0	1·62	0·37	0·19	0·03	0·13	0·22	0·6	0·6	−0·85

almost perfect regularity, a reduction both in the rate and the amplitude of the heart beat, whereas when the animal is deeply under (when the administration of the anæsthetic was pushed until the cardiac contraction was slightly affected) there is no, or at most merely the slightest, reduction in the rate and amplitude of the heart beat. That this inhibitory influence of the depth of the anæsthesia is a sensitive balance is shown by the fact that when a series of experiments are carried out in succession, beginning with the animal deeply anæsthetised and continuing without further administration of ether, there is a steady increase in the carbon dioxide poisoning effect as demonstrated by the gradual decrease in the amplitude of the heart contraction, particularly of the ventricle.

B. *Blood-pressure experiments.*

In view of the results obtained by Starling and his co-workers it was of interest to investigate the effect of the administration of carbon dioxide in varying depths of anæsthesia on the blood-pressure in addition to the heart direct.

Before determining the influence of the addition of the carbon dioxide we carried out a series of experiments on the direct influence of the anæsthetic alone on the blood-pressure. We found in a number of our experiments that on changing from "light" to "very deep" anæsthesia the blood-pressure might fall as much as 50 mm. of Hg. and that a corresponding rise in blood-pressure took place when the anæsthesia changed from "deep" to "light."

The effect of giving carbon dioxide was very marked in that, as the anæsthesia became less deep, the administration of the carbon dioxide air mixture brought about a gradual increase in the blood-pressure. In stages of light anæsthesia the carbon dioxide frequently caused a sharp rise of from 30 to 40 mm. Hg. from the base line at the commencement of the administration. Further, that the blood-pressure rise continued after the administration of the carbon dioxide had ceased, in other words that the apex of the blood-pressure rise was not synchronous with the point of minimum amplitude of the auriculo-ventricular trace. In deep anæsthesia this rise in blood-pressure was not brought about by the giving of carbon dioxide. On the other hand when the anæsthesia was less deep but still deep enough to prevent the carbon dioxide affecting very definitely the rate and the amplitude of the heart a rise in blood-pressure might occur.

As regards the relationship of the rise and fall in blood-pressure to the variation in the amplitude and rate of the cardiac contraction our experiments tend to show that, although there may be some connection, the diminished amplitude which results from the administration of carbon dioxide during light anæsthesia is not solely dependent upon the alteration of the blood-pressure. The following short summary and the subsequent protocols will serve to make these points clear.

Light anæsthesia + CO_2. Marked rise in blood-pressure. Labouring heart. Blood-pressure starts to rise before the heart trace becomes affected and the apex of the B.-P. curve is frequently subsequent to the point of minimum amplitude of the cardiac contraction; it may even occur after the cessation of the administration of the CO_2.

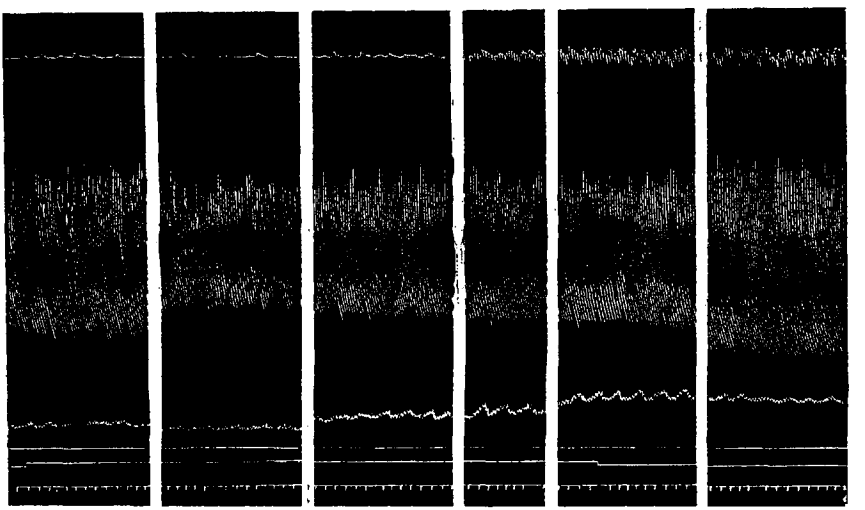

Fig. 5. Deep anæsthesia. Heart contraction and blood-pressure recorded simultaneously. 5 litres of 12 % CO_2-air mixture. Intervals = 14 secs. Total time = 111 secs.

Medium anæsthesia + CO_2. Rise in blood-pressure. No marked labouring of the heart although slight diminution in the amplitude may take place.

Deep anæsthesia + CO_2. No rise in blood-pressure. Practically no alteration of amplitude of the cardiac contraction.

The following experiments may also be cited:

Protocol III. The animal was very deeply anæsthetised and a series of tracings without further anæsthesia were taken at intervals of about

four minutes. (The experiment was stopped before the animal came out of the anæsthesia.)

(*a*) Animal deeply under. Five litres of 12% carbon dioxide air mixture given. The auricular contraction was poor before the carbon dioxide was administered but commenced to improve after about one minute and was quite good at the end of the administration. The ventricular trace showed a slight diminution in amplitude for some 25 seconds but subsequently increased to its initial size. The blood-pressure rose very slightly at first but not again until more than half of the carbon dioxide had been given when a second slight rise took place. In all the rise in blood-pressure from the beginning of the experiment until it reached its maximum height about 20 seconds after the cessation of the administration of the carbon dioxide was only about 12 mm. of Hg. (see Fig. 5). After the carbon dioxide was all given and pure air was administered the cardiac amplitude rapidly increased, but the blood-pressure after reaching its maximum very gradually fell away. The figures were:

Rate.	Commencement of experiment	Middle of experiment	End of experiment
Ventricle } in 3 seconds ...	13·0	13·0	13·0
Auricle } in 3 seconds ...	?	13·0	13·0
Amplitude.			
Ventricle } average of 3 contrac-	46·0 mm.	40·3 mm.	43·1 mm.
Auricle } average of 3 contrac- tions ...	?	1·0	3·0
Height of lowest point of ventricular contraction above the base line	76·0 mm.	84·0 mm.	80·0 mm.
Blood-pressure in mm. Hg. above base line	25·0 mm.	27·0 mm.	35·0 mm.*

* 30 seconds later 37·0 mm.

(*b*) No. 5 in series. Animal lightly anæsthetised and the heart beating well. When five litres of 12% carbon dioxide air mixture were given both the ventricular and the auricular contractions diminished in amplitude, the rate slowed but the blood-pressure rose some 30 seconds after the commencement of the experiment. After the administration of the carbon dioxide had ceased the heart began to contract better and the rate increased again, the blood-pressure however continued to rise for a further 30 seconds and reached its maximum at a point when the ventricular and auricular traces were rapidly increasing in amplitude (see Fig. 6). The figures were:

	Commencement of experiment	Middle of experiment	End of experiment
Rate.			
Ventricle } in 3 seconds ...	13·0	8·0	8·0
Auricle } in 3 seconds ...	13·0	8·0	8·0
Amplitude.			
Ventricle } average of 3 contractions ...	54·3 mm.	21·7 mm.	21·3 mm.
Auricle } average of 3 contractions ...	7·7	1·8	1·3
Height of lowest point of ventricular trace above the base line	78·0 mm.	101·0 mm.	101·0 mm.
Blood-pressure in mm. Hg. above base line	52·0 mm.	68·0 mm.	77·0 mm. *

* 30 seconds later, 90 mm.

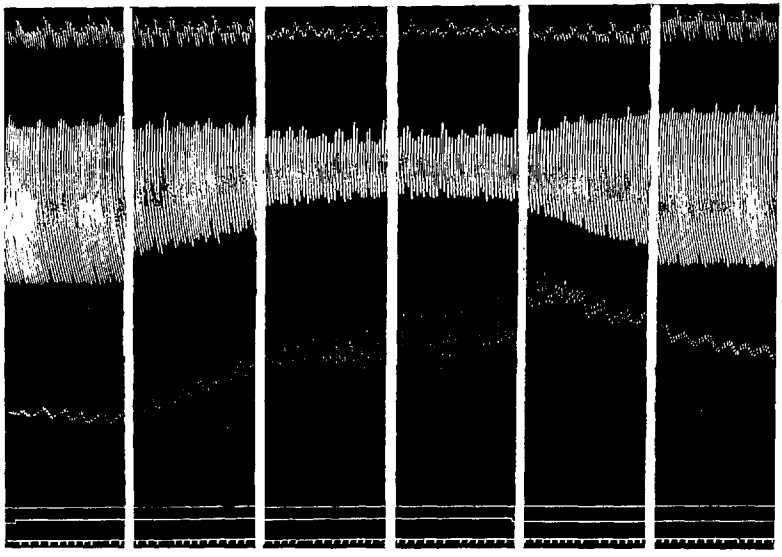

Fig. 6. Light anæsthesia. Heart contraction and blood-pressure recorded simultaneously. 5 litres of 12 % CO_2-air mixture. Intervals = 14 secs. Total time = 100 secs.

Thus it would appear that when the anæsthesia is deep, so deep that carbon dioxide has practically no effect on the amplitude of the heart beat, there is no marked rise in the blood-pressure in spite of the fact that the percentage of carbon dioxide used ought, if the explanation of Starling and von Anrep be the correct one, to have evoked a secretion of adrenalin with its accompanying rise in blood-pressure; and yet, with the light anæsthesia, when the amplitude is affected, we find the rise in blood-pressure noted by Starling. (It may be noted here that Elliot (18) has shown that ether itself does not have any direct effect in exhausting the suprarenals.)

At present we are carrying out a further series of experiments with a view to elucidating the manner in which the anæsthesia exercises its inhibiting action. Such results as we have obtained point to the inhibitory influence of the ether being due to its action on a central synapse although our experiments as yet are too few in number to permit us making at present any hard and fast generalisation.

As regards the practical aspects of our work it certainly gives support to the contention that if anæsthesia is to be carried out with any degree of safety it must be deep.

We wish to express our thanks to Mr A. Watson who kindly assisted at many of the experiments.

Part of the expenses of this research was defrayed by a grant from the Carnegie Trustees.

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CHLOROFORM ANÆSTHESIA IN THE LIGHT OF PHYSIOLOGICAL RESEARCH.

By GEORGE HERBERT CLARK, M.B., D.P.H.,
Lecturer in Physiology in the University of Glasgow.

(*Reprinted from the "Glasgow Medical Journal," January, 1914.*)

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CHLOROFORM ANÆSTHESIA IN THE LIGHT OF PHYSIOLOGICAL RESEARCH.[1]

By GEORGE HERBERT CLARK, M.B., D.P.H.,
Lecturer in Physiology in the University of Glasgow.

ANYTHING which throws light on the effects of chloroform anæsthesia is of practical interest, and this is particularly true of the dangers associated with the use of the drug. I venture, therefore, to communicate the following observations as they have, I believe, a very practical bearing on the subject.

During the summer of 1912 Dr. Cathcart and I commenced a series of experiments, in which we endeavoured to find out what was the effect of CO_2 in various percentages upon the heart, and whether the administration simultaneously of O_2 or lactic acid modified this. This work is at present being carried on, but somewhat unexpectedly our attention was directed to a fact that is of considerable clinical importance.

We found that when animals are *really* deeply under an anæsthetic the heart is very much less susceptible to the effects of CO_2 than when they are lightly under. When the animal is deeply under, the administration of CO_2 in varying percentages up to 20 per cent does not cause a marked effect upon the heart-beat for a very considerable time. When the animal is not deeply anæsthetised, however, soon after the administration of the CO_2 commences the heart-beat becomes slower and diminished in amplitude, the heart contracts less and less satisfactorily until perhaps the animal struggles in the first stage of asphyxia, or else the heart contraction

[1] A portion of an address delivered to the University Medico-Chirurgical Society in January, 1913.

becomes laboured, and may come almost to a standstill. This condition rapidly passes off on the circulation of pure air, or O_2, through the lungs. The fact has been tested by us repeatedly, and the result is always the same.

In all but one of the series of experiments which we have carried out the anæsthetic used was ether, and we are now endeavouring to obtain similar results with chloroform. Here we have been met with a somewhat unexpected difficulty, for rabbits, which we have used exclusively till now, do not take chloroform well. In these animals the rapid assumption of chloroform anæsthesia is followed by death. A gradually deepening anæsthesia, produced with very dilute $CHCl_3$ vapour is not so fatal, but it has a marked effect on the heart. We have found that the introduction of too strong a $CHCl_3$-air mixture kills the rabbit by throwing its heart into fibrillar contractions—that is to say, the normally co-ordinating mechanism is lost, and the various parts of each chamber contract, irrespective of any rhythm. The condition is generally fatal. We found, however, in the one experiment that we have done, that a CO_2-air mixture causes the heart to stop fibrillating, and to take on again a normal beat. What the exact reason for this is, is difficult to say, unless it is that the well-known stimulating action of CO_2 upon the vagus centre in the medulla causes a diminished excitability of the heart as a whole, and hence the pace-maker in the sino-auricular region can again take the lead.

The cause of death during chloroform anæsthesia has been very fully investigated by Levy, who shows quite conclusively that the period of danger is that of light anæsthesia. His general conclusions are sufficiently noteworthy to quote in full.

1. The mammalian heart, when under the influence of chloroform, is in an "irritable" (shows a tendency to exhibit beats of a heterogenic origin) condition. This irritability is raised under conditions of light anæsthesia, and lowered under conditions of deep anæsthesia.

2. Abnormal ventricular beats are evoked in a heart under chloroform by conditions which stimulate it, or by equivalent conditions which remove or reduce depressing influences.

3. Under conditions of light chloroform anæsthesia, the ventricular irregularities resulting from cardiac stimulation may terminate in ventricular fibrillation and death of the heart.

4. Stimulation of the heart may be effected—(*a*) as a reflex from sensory excitation; (*b*) as a result of an intermittent administration of the anæsthetic; (*c*) as a result of the state of nervous excitement, accompanied by struggling, induced by chloroform in the earlier stages of its administration.

5. Ventricular fibrillation is a cause of death under chloroform, probably the only cause of any moment. It can be prevented by steadily maintaining a full degree of anæsthesia.

A practical point of considerable interest is that adrenalin may produce fibrillation of the heart if administered in any quantity during light anæsthesia.

With chloroform, however, the great danger is not perhaps so much of an acute poisoning as of what is now described as delayed chloroform poisoning. This is a subject which has been investigated somewhat fully by physiologists and others, and as it has a very practical application in medicine and in surgery, I shall refer to one or two of the more important points in connection with it.

When any general anæsthetic is used, it owes its anæsthetic power to its ability to combine with lipoids, and more especially, in the first instance, with those of the red corpuscles. In a normal anæsthesia the distribution of chloroform in the blood is 88 per cent in the corpuscles and about 12 per cent in the plasma. During anæsthesia the corpuscles and plasma part with their chloroform to the tissues which possess lipoids, and more especially to those rich in these bodies, such as brain and nerve tissue. For this reason fat subjects require a large quantity of chloroform or other anæsthetic before they are anæsthetised, and they come out slowly after the anæsthetic is stopped.

The whole question of safety in anæsthesia is a question of rate of assumption and rate of elimination of the drug. The more rapid the rate of assumption the greater the danger; the more rapid the elimination the better for the patient.

The useful part of the anæsthetic appears to be that which attaches itself to the corpuscles, and the dangerous part, as far as delayed poisoning is concerned, that which passes, probably in solution, into the plasma. In any case, the amount in the corpuscles is rapidly assumed and rapidly eliminated, but that in the plasma is not so rapidly eliminated. This latter amount in all is, of course, small, and its effect on the tissues is correspondingly small, *unless it persists in the blood for some time.*

In cases which readily recovered from the anæsthesia, a

small moiety of chloroform is found in the blood some hours after the anæsthetic has been given. This quantity is much increased if the animal is kept quiet after the anæsthetic has been given, and during the time it is coming out. On the other hand, it is diminished with movement and free ventilation in the post-anæsthetic period.

When tissues are immersed in serum containing chloroform, a series of changes take place *in vitro* which are comparable with those which are found *post-mortem* in tissues removed from animals which have been anæsthetised with chloroform for a long time, or which have received doses by the stomach, and in which elimination is slow. These changes consist of granular degeneration of the cells of the kidney and liver. The most typical effect is the degeneration of the cells in the centre of the lobules of the liver. In marked cases almost the whole of the lobule undergoes degeneration, and a mere shell of healthy cells surrounds the degenerate tissue.

In these cases where this occurs elimination of the drug is found to be slow, and the blood invariably shows a high percentage of chloroform in the plasma. Thus, the rate of elimination appears to depend upon the distribution of chloroform between corpuscles and plasma. In those animals whose plasma takes up a much larger proportion of chloroform than normal the symptoms of delayed chloroform poisoning are found.

The destruction of the centre of the lobules of the liver profoundly modifies the metabolism of the animal, and loss of weight, vomiting, diarrhœa, acidosis, albuminuria, and eventually death, occur. Along with this, the destruction of the liver tissue, according to Hagan and Ormond, results in a reduced production of fibrinogen, and therefore a delayed clotting of the blood. This delay in clotting of the blood is probably the cause of reactionary hæmorrhages after operations. As recovery takes place the liver lobules regenerate, the fibrinogen production increases, and the metabolism gradually becomes normal. This, however, does not take place in a very large proportion of all the cases.

Where small doses of the drug are given by the stomach over long periods, the result is also to produce a form of chronic chloroform poisoning with somewhat similar symptoms. While the drug is not generally given in this form, it is taken in the form of chlorodyne in lozenges over long periods, particularly, as was pointed out by Crichton Browne, in certain districts in England. Animals subjected to this

treatment over short periods die with extensive degenerative changes in the liver, and sometimes in the kidneys and heart.

So far there appears to be no means of finding out before administration of an anæsthetic whether the plasma of the patient will take up a smaller or larger percentage of chloroform. As it is, the first symptom is a continued vomiting, which may persist for a week or more after the anæsthetic has been given.

These cases, when they occur, should be treated in such a way as to increase the elimination of the drug and thus get rid of it as soon as possible, and in that way allow repair of degenerative tissue to take place. Thus lots of fluid and plenty of fresh air are of service. To allow a patient to "sleep off" chloroform is not a good thing if he shows signs of sleeping for a long while; so also as regards administration of chlorodyne, or any form of chloroform, by mouth. This should never be continued for long in any condition.

Before leaving this subject, I may remark upon the idea that is generally held that pregnant animals, and patients in course of being delivered, are almost immune to chloroform poisoning. Experimental work has shown this to be a fallacy, as pregnant dogs and cats are somewhat *more* susceptible to the action of the drug. Curiously enough, the fœtus is not susceptible to chloroform poisoning, in dogs at least, until three weeks after birth.

Chloroform, then, is a drug which, while it is admittedly handy and easily given, is dangerous during its administration when heart failure is liable to occur from various causes; it is dangerous afterwards because it is liable to cause a train of symptoms known as delayed chloroform poisoning, which is not by any means so uncommon as is supposed. Its use as an anæsthetic should be discouraged, and the habit of giving small doses to relieve pain which is not likely to be severe cannot be too strongly condemned. The mere fact that the drug is being given to relieve a paroxysm of pain often leads to its being administered very rapidly and often without due care. The rapid and irregular administration of such a drug is most frequently the cause of death from heart failure when death occurs.

When chloroform is administered, every care must be taken to ensure that the rate of administration is slow, and the rate of elimination rapid. I am conscious of the fact that I am making out quite the worst case against chloroform, and

that many individuals who use it very freely claim to have had no accident from early or late chloroform poisoning. My reply to that is that I have known of several cases of late—cases which have occurred since I have been specially interested in this subject—which have died at varying times after operation, from symptoms identical with those of delayed chloroform poisoning. In some of these cases, where death had been described as due to "shock," *post-mortem* examination showed changes in the liver, such as occur in delayed chloroform poisoning.

[Grants from the Carnegie Trust were received to defray the expenses of the author's researches referred to above.]

ON THE DANGERS OF LIGHT ANÆSTHESIA.

By G. H. CLARK, M.B., D.P.H.,
AND
E. P. CATHCART, M.D., D.Sc.

(*Reprinted from the "Glasgow Medical Journal," April, 1914.*)

increased CO_2 content of the blood stimulated the secretion of adrenalin. In this connection the further observations of Cannon and Hoskins[1] are of considerable interest and importance. These workers found that sensory excitation, nervous excitement, fear, all could stimulate the secretion of adrenalin. Finally, Levy showed that another factor of great importance in the production of cardiac irregularities was the method of administration of the anæsthetic. If it were given in an irregular and intermittent manner there was grave danger, owing to sudden changes in the heart from a condition of hyper- to hyposensitiveness, and *vice-versa*—a state of affairs which rendered the heart very subject to the induction of irregularities.

We[2] have recently shown that, in the case of the rabbit, using ether as the anæsthetic, the action of CO_2 on the heart and circulation is practically *nil* when the anæsthesia is deep, but is very marked when the anæsthesia is light. In other words it would seem that during deep anæsthesia the extra secretion of adrenalin as the result of CO_2 stimulation does not take place. Although the heart and blood-vessels do not respond to CO_2 in deep anæsthesia, this is not due to inability of the vessels to respond, as we have shown that in anæsthesia, pushed until the heart became affected, the injection of adrenalin could still produce its characteristic effect upon the heart and blood-vessels.

In our experiments we investigated the action of the CO_2 in three different ways—(1) on the heart-rate, (2) on the amplitude of the cardiac movements, and (3) on the blood-pressure. We found that with light anæsthesia the average decrease in the rate during the administration was 1·75 contractions in three seconds, and in amplitude there was a diminution of 13·89 millimetres. With deep anæsthesia, on the other hand, the average decrease in rate was only 0·19 contractions in three seconds, and in amplitude 0·85 millimetres. As regards blood-pressure, we found that in deep anæsthesia there was little or no rise, whereas in light anæsthesia the administration of CO_2 brought about a very marked rise. This difference is well shown in the tracings given below.

The following record of one of our experiments illustrates well the various points:—

A rabbit was deeply anæsthetised with ether, and, without further administration, was allowed to pass gradually from

[1] Cannon and Hoskins, *Amer. Jour. of Physiol.*, vol. xxix, p. 274.
[2] Cathcart and Clark, *Jour. of Physiol.*, 1913, vol. xlvii, p. 393.

before attempting to explain the cause of the danger of light anæsthesia, a few facts of physiological importance must be referred to.

the state of deep to that of light anæsthesia. Observations were made every four minutes. (*a*) Animal deeply under (No. 2 of the series of observations). Five litres of a mixture of air, containing 12 per cent of CO_2, were given, and the effects recorded. The tracing below (Fig. 1) shows the changes that took place in the heart (top line auricle, second line ventricle), and blood-pressure (third line). The bottom line shows time in seconds, and the next marks, by up and down strokes, the beginning and the end of the administration of the CO_2. The auricular contractions are seen to improve

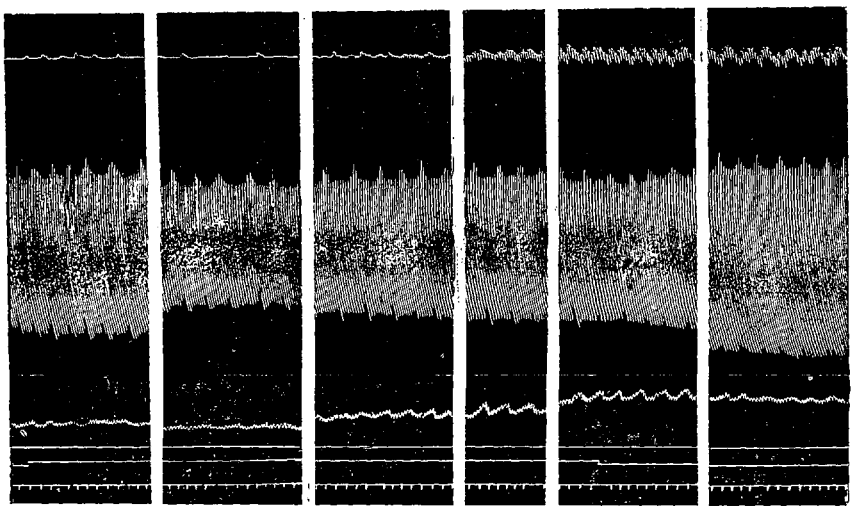

FIG. 1.

Deep anæsthesia. Heart contraction and blood-pressure recorded simultaneously. 5 litres of 12 per cent CO_2-air mixture. Intervals = 14 secs. Total time = 111 secs.

throughout the administration. The ventricular contractions diminished slightly at first, but subsequently returned almost to their initial size. The blood-pressure rose very slightly at first, and again slightly towards the end of the administration. In all, the greatest increase in the blood-pressure in this experiment only amounted to about 12 millimetres of Hg. After the CO_2 was all given, and pure air substituted, the heart rapidly returned to normal. The blood-pressure only returned slowly to its previous level. (Note.—As animals become less deeply anæsthetised, the blood-pressure progressively rises, and *vice-versa*.) The figures were :—

Rate.	Commencement of experiment.	Middle of experiment.	End of experiment.
Ventricle } in 3 seconds	13·0	13·0	13·0
Auricle } in 3 seconds	?	13·0	13·0
Amplitude.			
Ventricle } average of 3 contractions	46·0 mm.	40·3 mm.	43·1 mm.
Auricle } average of 3 contractions	?	1·0	3·0
Height of lowest point of ventricular contraction above the base line ...	76·0 mm.	84·0 mm.	80·0 mm.
Blood-pressure in mm. Hg. above base line ..	25·0 mm.	27·0 mm.	35·0 mm.*

* 30 seconds later, 37·0 mm.

(*b*) No. 5 in the series. The animal was now only lightly

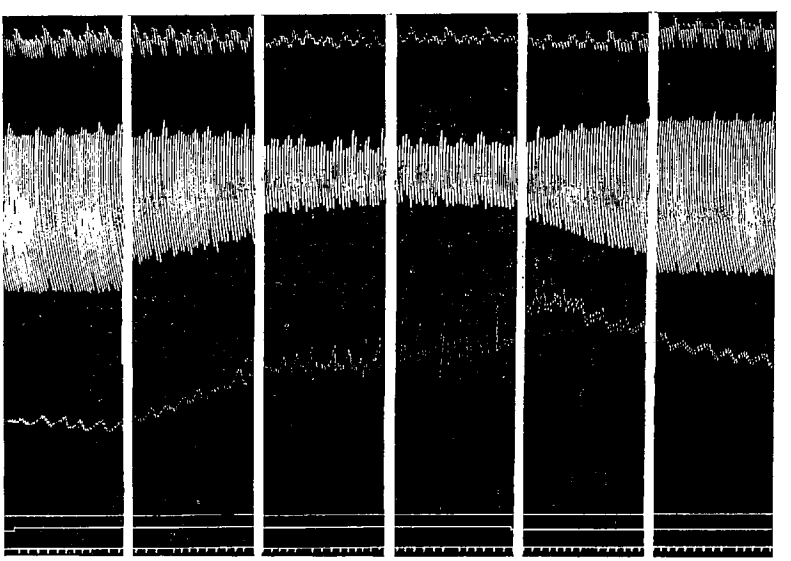

FIG. 2.

Light anæsthesia. Heart contraction and blood-pressure recorded simultaneously. 5 litres of 12 per cent CO_2-air mixture. Intervals = 14 secs. Total time = 100 secs.

anæsthetised. The heart was beating well. A similar quantity of the CO_2-air mixture was again given. In Fig. 2

the result is shown. Both the auricular and the ventricular amplitude rapidly diminished, the rate slowed, and the blood-pressure commenced to rise some thirty seconds later. After the CO_2-air mixture was all given, the heart amplitude rapidly increased to normal, but the blood-pressure only gradually came down. The figures were:—

	Commencement of experiment.	Middle of experiment.	End of experiment.
Rate.			
Ventricle } in 3 seconds	13·0	8·0	8·0
Auricle } in 3 seconds	13·0	8·0	8·0
Amplitude.			
Ventricle } average of 3 contractions	54·3 mm.	21·7 mm.	21·3 mm.
Auricle } average of 3 contractions	7·7	1·8	1·3
Height of lowest point of ventricular trace above the base line	78·0 mm.	101·0 mm.	101·0 mm.
Blood-pressure in mm. Hg. above base line ...	52·0 mm.	68·0 mm.	77·0 mm.*

* 30 seconds later, 90 mm.

We consider that it is now proved experimentally that safety lies in deep, and danger in light, anæsthesia; that continuous, and not intermittent, administration is essential; and that the use of adrenalin in any form during anæsthesia is fraught with danger.

[Grants were received from the Carnegie Trust towards the expenses of the authors' research referred to.]