THE CAUSATION OF DEATH DURING THE ADMINISTRATION OF CHLOROFORM.

By E. H. EMBLEY, M.D.,

Honorary Anaesthetist to the Melbourne Hospital.

(From the Physiological Laboratory of the University of Melbourne.)

PURPOSES OF THE INVESTIGATION.

To those in the habit of administering and supervising the administration of chloroform, the physiological investigations hitherto made into the action of chloroform upon the organism have not furnished a satisfactory explanation of the phenomena that are apt to occur during the induction or early period of anaesthesia in chloroform administration.

Efforts have frequently been made towards reconciling clinical experience with the results of physiological research. These have been too frequently in the direction of belittling clinical experience by declaring that when clinical experience failed to coincide with the results of experiments upon animals, clinical observation was erroneous.

It was evident to clinicians that the heart did stop in occasional instances when the respiration was efficiently performed, and that in some of these cases the heart started again, in others it did not. It was also evident that early heart failure was rarely, if ever, attended by a regular falling off to imperceptibility in pulse volume, but that when observed the stoppage of the pulse was sudden and absolute, and the recovery, when such happened, was attended by a slow pulse of good volume. It was therefore difficult in view of these observations to reconcile the respiratory failure theory as the cause of all deaths from chloroform. It was equally difficult to conceive that vasomotor paralysis could be responsible for the failure of circulation, as the first heart beats in cases which recovered often gave a fair to good tension pulse.

The investigation of the cause of death in early anaesthesia was chosen for particular research in consequence of the greater tendency to death at this period. The greater frequency of deaths before the surgeon has started to operate has been well recognized. During 1899, out of 83 cases of death that occurred in England, 68 happened before the operation was started.² In another year it was 39 out of 41.¹ My own experience at the Melbourne Hospital has convinced me that the period of early anaesthesia by chloroform is the period of by far the greatest danger.

The research was carried out at the Physiological Laboratory of the University of Melbourne. To Professor C. J. Martin I am under great obligations. It is in consequence of his generous assistance and advice and his aid in designing new apparatus, that I have been enabled to carry out the investigation.

HISTORICAL REVIEW.

The diversity of opinion resulting from previous physiological investigations into the question of the causation of death from chloroform may be gathered from the brief outline given below.

Snow³ found that the respiration failed before the heart when low percentages of chloroform vapour in air were employed, and that the heart may cease before the respiration when high percentages were administered.

Ringer⁴ found that the heart of the frog passed into a state of paralytic dilatation when chloroform was introduced into the circulation.

The Glasgow Committee⁵ concluded that retardation of the heart's action occurred under and in recovery from chloroform, and that stoppage of the heart from this cause was dangerous. The Committee were led by these effects upon the heart to say that the action of chloroform was capricious. They⁵ also observed that respiration sometimes continued after the blood pressure had fallen to zero.

Wood⁶ concluded that the heart may be paralysed by chloroform, and that respiration may continue for some time after circulation ceased.⁶ He found that chloroform abolished the power of the circulatory mechanism of adjusting for the influence of gravity in altered positions of the body.

MacWilliam⁷ found that chloroform always caused dilatation of the heart "even with low percentage vapour," whether the vagi were cut or not; that the fall of blood pressure was caused by vasomotor and cardiac paralysis combined; that respiration generally ceased before circulation failed, but that the circulation at times failed first, in which case respiration continued for some time after circulation ceased; that slowing of the heart occurred from vagus action, and that it was not a source of danger.

The Hyderabad Commission⁸ conclude from their results that interference with or paralysis of respiration is the only means by which the heart's safety is jeopardized; that in ordinary inhalation the chloroform is never taken in sufficient quantity to stop the heart before the respiration. They⁸ also formed remarkable theories regarding vagus inhibition. They credit Dr. Bonford with the "great discovery" that "stoppage of the heart by vagus action is a natural safeguard against the distribution of chloroform, and that it is therefore beneficial." They deny that chloroform impairs the heart. They think that the fall of blood pressure from chloroform is from vasomotor paralysis, and is beneficial to the heart. They declare that the effects of the Glasgow Committee were asphyxial, and they impute defective methods and erroneous inference to the Committee.

Hare and Thornton⁹ concluded that the fall of blood pressure from chloroform was from vasomotor paresis and heart paralysis combined, with the former as the chief factor; that⁹ vagus inhibition was never sufficient to permanently stop a sound heart, but that it may stop a diseased heart in frightened persons, with the assistance of vasomotor paralysis; that⁹ heart failure never occurs before respiratory failure.

Dastre¹⁰ states that the syncope which occurs occasionally in man and animals in the beginning of the chloroform administration is due to reflex stimulation of the pneumogastric nerves, and emphasizes the trigeminal (the nasal mucous membrane) and the superior laryngeal (larynx) as sensory nerves, stimuli from which are particularly prone to occasion fatal reflex inhibition of the heart during the early period of anaesthesia. Dastre and Morat¹¹ found that postponement of death is effected by using atropine or by section of the vagi. This they thought to be due to the abolition of vagus reflexes. They considered that cessation of the respiration is one cause of fatal arrest of the heart. Dastre argues that chloroform causes vaso-constriction.

Morat and Doyen¹¹ find that the principal danger of chloroform is during the induction, and that it results from a tendency to syncope by arrest of the heart; that this is due to the excitation of the pneumogastric nerves; that the excitability of these nerves before it is diminished by chloroform along with the rest of the nervous mechanism is temporarily exalted.

Gaskell and Shore¹² concluded from the results of their research that the fall of blood pressure in chloroform narcosis was caused by heart failure alone; that the vasomotor system was not paralysed, but actually stimulated; that the fall of blood pressure was dangerous; that the heart may fail before the respiration, and that respiratory failure was mainly determined by the fall of blood pressure. They¹² did not think that chloroform produced local paralysis of the arterioles.

Richet¹³ concludes that respiratory failure is never dangerous as long as the heart continues to beat, if artificial respiration is resorted to; that asphyxia never causes death of the heart in less than four or five minutes, and he considers it absurd that a patient should be supposed to remain unobserved without breathing for all that time. He states in support of this contention that a "chloroformed animal supports asphyxia as well as, if not better than, a normal animal." He goes so far as to state that "death never occurs by failure of respiration." He concludes that danger of death is solely cardiac; that when cardiac syncope occurs, artificial respiration never restores it, and cardiac syncope results from a profound intoxication of the myocardium or the intrinsic ganglia. As regards reflex syncope from the stimulation of sensory nerves, he¹⁴ finds that slowing of the heart occurs, and possibly actual syncope, but he does not believe that such syncope is ever fatal under chloroform.

Hill¹ concluded that the fall in blood pressure was due to

vasomotor paralysis combined with heart paralysis; that¹ the fall in blood pressure was the greatest factor in the causation of respiratory failure, that is, that circulatory failure was the cause of respiratory failure, even though the respiration may have failed before the circulation ceased; that¹ vagus inhibition was never sufficient to permanently arrest the heart.

Waller¹⁵ made some observations on safe and unsafe percentages of chloroform by experiments upon isolated nerve, and found that the effects produced by different percentages of chloroform in the air upon the excitability of nerves, led him to the same conclusions as Snow,³ Paul Bert,²² and Dubois,²³ namely, that 2 per cent. chloroform vapour in the air is too much, and that 1 per cent. to 1.5 per cent. is sufficient to maintain anaesthesia by continuous inhalation.

Further research by MacWilliam⁷ has led him to conclude that the cardio-inhibitory mechanism is very excitable in the early stage of chloroform anaesthesia. He found that the excitability varied in different animals. He obtained reflex inhibition of the heart, which, however, was not fatal. He found that during deep chloroform anaesthesia the inhibitory mechanism was still irritable.

Duplay and Hallion,¹⁶ investigating the time relations of respiratory and circulatory failure, concluded that deaths from chloroform were never due to failure of respiration; that the cause of syncopal deaths was cardio-vascular paralysis, and that this happens about the same time as respiratory failure.

The Hyderabad Commission⁴ were unable to obtain fatal inhibition of the heart by vagal stimulation under chloroform anaesthesia.

Hill¹ was unable to obtain fatal inhibition of the heart from chloroform anaesthesia alone.

Brodie and Russell¹⁷ obtained reflex cardiac inhibition from the stimulation resulting from the administration of chloroform vapour by respiration. The reflexes were found to result from the stimulation of the nasal, laryngeal, and tracheal mucous membrane, and especially from the pulmonary alveoli.

The Committee appointed by the British Medical Association¹⁸ to investigate the clinical evidence regarding anaesthetics concluded that "when danger occurs under chloroform, whatever its exact nature may be, there is abundant evidence that in a large proportion of cases the symptoms that are observed are those of primary circulatory failure."

These different views are more briefly stated in the following synopsis:

1. Chloroform has a paralytic effect upon respiration.—All.
2. Chloroform causes a fall of blood pressure.—All.
3. Respiratory failure the cause of circulatory failure.—The Hyderabad Commission and Dastre (one of the causes).
4. Respiratory failure mostly the result of circulatory failure.—Gaskell and Shore, MacWilliam, the Glasgow Committee, and Hill.
5. Uncomplicated respiratory failure never causes death if artificial respiration is resorted to.—Richat and Duplay and Hallion.
6. Respiration always fails before the circulation.—The Hyderabad Commission and Hare and Thornton.
7. Circulation sometimes ceases before respiration.—Snow, the Glasgow Committee, MacWilliam, Gaskell and Shore, Wood, Hill, Richet, Dastre, Duplay and Hallion, Morat and Doyen.
8. Fall of blood pressure due to heart failure alone.—Wood, Gaskell and Shore, Richet.
9. Fall of blood pressure due to vasomotor paralysis alone.—The Hyderabad Commission.
10. Fall of blood pressure due to vasomotor and heart paralysis combined.—MacWilliam, Hare and Thornton, Hill, Duplay and Hallion.
11. Vasomotor system [not paralysed].—Gaskell and Shore, Dastre.
12. Vagus inhibition never causes death.—MacWilliam, the Hyderabad Commission, Wood, Hill, Richet.
13. Vagus inhibition dangerous.—Glasgow Committee, Hare and Thornton (conditionally), Dastre, Morat and Doyen.
14. Reflex vagal cardiac inhibition produced by inhalation of chloroform.—Dastre and Brodie and Russell.

The Hyderabad Commission report a large number of accidental deaths during their experiments.²⁶ Nine deaths

out of 154 experiments occurred in the period of early anaesthesia.²⁶ They attributed these occurrences to neglect in administration. There is no tracing furnished of them. This similarity to the fatalities in surgical cases does not appear to have attracted the attention of the members of the Commission, otherwise they would scarcely have completed their investigations without endeavouring to obtain tracings of this dangerous period of the administration. Their dogs were generally anaesthetized by chloroform in a box,²⁶ then taken out and operated upon under this anaesthesia, for connexion with the recording apparatus. The graphic records of the Commission, therefore, only represent the comparatively safe period of chloroform administration in dogs. The Commission were unable to inform us concerning the interesting phenomena that occurred in the box.

The tracings of the Hyderabad Commission as far as they go, no doubt represent the succession of events that occur in the deepening narcosis, in this later and comparatively safe period of chloroform anaesthesia, but their inferences from these results to the sudden deaths of early anaesthesia of surgical cases are invalid. Transient recoveries and reinductions, as will be shown later, by no means represent the conditions of the primary induction. The experiments which the Commission performed without graphic records, though including the induction period, were faulty and misleading in consequence of the defective methods²⁶ of observation, as was clearly pointed out by Hill.¹

Hill, Dastre, and Morat and Doyen have drawn attention to the fact that fatalities usually occur, in surgical cases, in the early period of anaesthesia, and each has suggested an explanation, but they do not seem to have experimentally investigated the matter.

It does not appear that either Gaskell and Shore, Hare and Thornton, Wood, the Glasgow Committee, MacWilliam or Richet paid any more attention to the dangers of early anaesthesia than did the Hyderabad Commission.

METHODS OF EXPERIMENT.

The details of experimental methods will be dealt with in treating of the various sections, but those methods which have been generally applied may be briefly mentioned here.

ARTIFICIAL RESPIRATION.

In experiments in which artificial respiration was employed this was effected by means of the Cambridge Scientific Instrument Company's apparatus.

APPARATUS FOR REGULATING THE PERCENTAGE STRENGTH OF CHLOROFORM VAPOUR IN THE AIR USED IN EXPERIMENTS WITH ARTIFICIAL RESPIRATION.

In most previous experimental work a knowledge of the exact strength of chloroform in the air which was delivered to the animal has not been forthcoming. Snow,³ Paul Bert,²⁰ and Dubois¹⁹ ascertained this with accuracy, but their experiments were directed to discovering what was the net effect of different quantities of chloroform, and not to an analysis of the phenomena.

In the present work percentages of chloroform vapour in the air were regulated in the following way: Air coming from the artificial respiration apparatus passed through tube A (Fig. A), whence it reached A' by passing through B and C. C has a constant resistance E. In the course of B was placed the chloroform bottle, also a variable resistance D in the form of a screw tap provided with a graduated dial. The proportion of the total air passing over the chloroform was regulated by varying the resistance in B. The air passing along B was dried in the sulphuric acid bottle S to prevent condensation of water on the chloroform. The combined air and chloroform vapour was then warmed in the bath H before reaching the dog by the tracheal tube I. By means of the resistance tap K efficient aëration was attained for small and large dogs alike. The total volume of air and chloroform vapour was then measured by the meter M.

To ensure regular volatilization of the chloroform, its temperature had to be maintained constant. To do this the chloroform cylinder was constructed of copper, as being the best conductor of heat available, and immersed in a water bath and maintained at a constant temperature by means of a thermostat.

Fig. A.—A', delivery tube from artificial respiration apparatus; B B B, loop tube; S, drying bottle containing H_2SO_4 on pumice; D, screw tap fitted with graduated dial; E, resistance tube; F, copper Woulff's bottle containing the chloroform; G, water bath; H, bath for heating air delivered to dog; I, trachea tube; K, resistance tap for adjusting the apparatus for different sizes of dogs; M, meter.

The value of the graduations on the dial of the screw-tap in terms of percentage of chloroform in the air administered was ascertained by passing air through the apparatus, and through the meter for ten minutes at a time, with the tap opened to different degrees 10, 15, 20, etc. The chloroform bottle was weighed before and after the passage of the air. The difference gave the weight of the chloroform which had been evaporated. The volume of the combined air and chloroform was read on the meter. Chloroform vapour corresponding to the weight of the chloroform evaporated was calculated by taking 1 gram of chloroform as affording 200 c.cm. of vapour at the temperature and pressure employed.

Having found the value of some of the divisions of the tap scale in terms of percentage chloroform in the air, these were plotted as ordinates against the corresponding divisions of the tap scale along the abscissa and the curve Fig. B drawn.

Fig. B.

From this curve the value of the intermediate positions of the tap scale can at once be found by interpolation.

This was done : —

(1) With the temperature of the chloroform at 20° C.

(2) With the temperature of the chloroform at 30° C.

For ordinary purposes the chloroform bottle was at 20° C., but when high percentages of vapour were required 30° C. was necessary.

To start the administration of chloroform the screw tap was set for the percentage required and the clamp removed from the tube on the proximal and distal sides of the chloroform bottle. To ensure greater accuracy the chloroform bottle was weighed both before and after the experiment, and the meter read at the same times. The percentages were thus actually determined for each separate experiment.

APPARATUS FOR ADMINISTERING KNOWN PERCENTAGES OF CHLOROFORM IN AIR IN NATURAL RESPIRATION EXPERIMENTS.

For this purpose the meter was placed between the tube A (Fig. A) and the warming apparatus H. Through H a stream of chloroformed air was delivered into a dry gasometer.

The dog breathed from the gasometer. The expired chloroformed air was directed into the external air by gut valves. The surplus escaped from the gasometer by a valve.

The apparatus was always set working some time before the experiment began, in order that the water of the meter might attain the same vapour tension of chloroform as that of the air passing through it. In this manner possible error arising from the water of the meter abstracting chloroform from the air administered to the dog was obviated.

RECORDING APPARATUS.

The mercurial manometer was used for most of the blood-pressure tracings. The rubber (Von Frey) manometer was used where long tracings were required.

The Von Frey manometer was calibrated against a mercurial manometer. It was found that for the range used the excursions of the lever were directly proportional to the pressure, and that 49 mm. excursion of the lever of the Von Frey manometer equalled 200 mm. of mercury pressure, or, roughly, 1 mm. of the former equals 4 mm. of mercury pressure.

PRESENT INVESTIGATION.

Particular attention was devoted to recording changes in the circulation during the early period of chloroform administration in animals which had not previously received chloroform. This was accomplished in two ways:

1. By performing all operative procedures necessary for studying the circulatory changes under morphine narcosis, either alone or with curare.

2. By inserting the cannulae, etc., under ether anaesthesia, and eight or twelve hours after, when the effects of the anaesthetic had passed off, connecting with the recording apparatus. The chloroform was administered off a towel.

In consequence of the known liability of dogs to die suddenly in the early stage of chloroform anaesthesia, when the chloroform is given by the towel method—a liability to which human beings are also subject in a less degree—these animals were chosen for the experiments.

In the whole investigation 289 dogs were employed, and 284 graphic records were obtained.

I.—THE EFFECTS OF CHLOROFORM UPON THE HEART ISOLATED FROM THE CENTRAL NERVOUS SYSTEM.

Blood-pressure tracings of the effect of chloroform upon the heart isolated from vagus, vasomotor, and respiratory influences, were obtained by the method of Hering.²⁴

By this method the clamped carotid artery is connected directly with the clamped jugular vein by means of a U cannula, the other carotid being used for recording the blood pressure. The aorta is then ligatured just beyond the left subclavian artery, and when the blood pressure has become constant the artificial circuit is unclamped and the two subclavian arteries tied proximal to the vertebrae. The vagi are cut and artificial respiration is then started. When the blood pressure again becomes constant the chloroform is administered.

Nineteen experiments were performed with the isolated heart. Tracing 1 is a typical blood pressure tracing by Hering's method, of the effect produced upon the heart by chloroform. The other tracings differ only in the steepness of the fall in blood pressure, the degree of steepness depending upon the percentage strength of the chloroform used. In tracing (1) the chloroform vapour in the air inhaled was 1.2 per cent. The blood pressure is seen to commence to fall almost coincidentally with the administration. The high blood pressure at which the heart was working when the administration was started makes the former part of the fall in blood pressure more marked than the latter. There was no preliminary small rise of blood pressure such as is usually seen in experiments on the intact animal.

The heart is very sensitive indeed to the action of chloroform. When there was in the air inspired 0.8 per cent. chloroform vapour, blood pressure fell to zero in 16 min. 22 sec.; with 1.2 per cent. chloroform vapour, blood pressure fell to zero in 9 min. 45 sec.; with 2.0 per cent. chloroform vapour, blood pressure fell to zero in 1 min. 25 sec. The rapidity with which these quantities (0.8 to 2 per cent.) paralysed the isolated heart was surprising, for so far I have not succeeded in killing an intact animal by artificial inhalation and the vagi cut, with 0.8 and 2 per cent. in less time than 5 to 6 and 1 to 2 hours respectively.

The amount of circulating blood in Hering's method of performing this experiment is very limited, probably not more than

Tracing 1.—(Isolated heart, recovery.) Dog, weight 8 lbs. Morphine, 0.25 gram; 0.7 per cent. solution of chloroform in normal saline at 40° C. injected into brain by internal carotid artery for the anaesthesia during operation. Hering's method for artificial circulation. Vagi cut. Artificial respiration. Blood-pressure tracing. Blood pressure at starting equalled 212 mm. of mercury. Chloroform, 1.2 per cent., in inspired air at "on" continued for 45 sec. and stopped at "off." The fall of blood pressure that occurred equalled 88 mm. of mercury. In recovery blood pressure rose again to 180 mm. of mercury in 16 min. 40 sec. from time of cessation of administration. The drum was stopped four times for three minutes each during recovery.

← 1.2% CHCl_3 in inspired air

← CHCl_3 stopped.

Abcissa raised 30 mm.

Fig 1

3 Minutes interval
at each step

one-tenth* of the animal's blood being contained in the circuit.

That 0.8 per cent. of chloroform vapour in the inspired air produced paralysis of the heart under these circumstances in 16 min. 25 sec. must be due to the rapid rise in vapour tension of chloroform in the small and isolated quantity of circulating blood. This small quantity of blood cannot give its chloroform to the tissues and lymph. For the same reason the rapidity of recovery when chloroform administration was suspended in these experiments corresponds to the rapidity with which the heart is affected. This is seen in tracing (1). No sudden failure occurs from the action of chloroform on the isolated heart. The fall of blood pressure is gradual or steep according to the percentage of chloroform vapour in the air administered, but there were no abrupt variations in the rate or efficiency of the heart such as occur in an animal with an intact nervous system.

EXPERIMENTS WITH THE HEART ONCOMETER.

Twelve experiments were performed with the view to recording the changes in the volume of the heart produced by chloroform administration. The instruments used were air oncometers. Several sizes were necessary to accommodate different hearts. It consisted of a hollow metal vessel, larger than the heart for which it was used. The top was closed, with the exception of a small opening into which was let the tube for connexion with the tambour. The bottom was open, but constricted into a neck with a turned-out lip. The opening in the neck was about two-thirds the diameter of the vessel.

In applying the oncometer to the heart, the sternum, with about 3 inches of rib from each side, was removed, after the intercostal and internal mammary arteries had been secured. The pericardium was then opened wide enough to admit the neck of the oncometer. The heart was next raised into the vessel, and the pericardium drawn up firmly and secured around the neck of the oncometer by an elastic band. The heart was thus enclosed in an almost unyielding chamber, the interior of which was in communication with the tambour. Artificial respiration was, of course, necessary throughout. Simultaneous records of arterial blood pressure were taken.

The heart was found to undergo rapid dilatation when the air respired contained 3 per cent. chloroform vapour and upwards. Slower dilatation occurred when lower percentages were employed; at the same time marked diminution occurred in the contraction volume *pari passu* with the fall of blood pressure. Dilatation happened, also, when the vagi were cut. The diminution in the contraction volume tailed off to imperceptibility as the blood pressure reached zero. When the vagi were intact, however, inhibition often terminated the circulation suddenly, and both tracings simultaneously ceased.

These results correspond with those of MacWilliam.⁷

CONCLUSIONS REGARDING THE EFFECTS OF CHLOROFORM ON THE HEART ISOLATED FROM THE CENTRAL NERVOUS SYSTEM.

1. Chloroform has an immediate and progressively paralytic effect upon the heart muscle. There is no preliminary period of stimulation. There is no abrupt change in the rate or efficiency of the heart.

* Six attempts were made to determine this quantity. They gave widely varying results. One estimation gave as high as one-sixth and another as low as one-sixteenth of the total blood. The lowest value was obtained in an experiment in which the heart was vigorously contracting prior to the ligaturing off, and continued to beat well afterwards, driving the blood from the left to the right side through the coronaries only; the highest value when the heart was dilated and beating feebly before the last ligature was tied. These estimations were effected in the following manner: The morphinized dog was curarized. A standard was then made by diluting 1 c.cm. of the dog's blood with 99 c.cm. of distilled water. Artificial respiration was then commenced. The thorax was next opened by section along the sternum. The exposed aorta was tied close up to the left subclavian artery. The inferior vena cava and the azygos vein were next tied. The left subclavian was next tied, followed by the trunk of the right subclavian and carotids, simultaneously with the superior vena cava. The lungs and heart were then removed, and water run through from the inferior vena cava and out by the aorta until the washings came out colourless. The washings were then measured, and 10 c.cm. taken and diluted to match the colour of the standard. From the data thus obtained the amount of dog's blood in this portion of the circulation in relation to the total blood was readily calculated. The total blood was assumed to be one-thirteenth of the body weight.

2. Heart muscle is very sensitive to the poisonous effect of chloroform—a tension of chloroform in blood corresponding to 0.8 per cent. chloroform vapour in the air inhaled kills the isolated mammalian heart in sixteen minutes.

3. In the administration of chloroform by inhalation to the intact animal, the vapour tension of chloroform in the blood only slowly reaches that of the inspired air.

(To be continued.)

A CONTRIBUTION TO THE STUDY OF THE PRESENCE AND FORMATION OF AGGLUTININS IN THE BLOOD.

By M. ARMAND RUFFER and M. CRENDIROPOULO,

M.A., M.D. Oxon.,
President of the Sanitary,
Maritime, and Quarantine
Council of Egypt.

M.D.,
Medical Director (1st class)
Sanitary, Maritime, and
Quarantine Council of Egypt.

(From the Port Vieux Laboratory, Alexandria.)

THE origin in the animal organism of substances antagonistic to microbes has for a long time attracted the serious attention of observers, and many have endeavoured to solve a question which from so many points of view is so intensely interesting. Although special researches with a view to localizing the origin and the formation of agglutinins in the animal organisms are numerous, observers unanimously recognize that of all organs of the body the blood is richest in agglutinins, though these peculiar substances are also present in the lymph, in the pericardial and pleuritic fluids, and even in oedematous exudations which contain but very few cellular elements.

PREVIOUS RESEARCHES.

Nevertheless, it is by no means clear in what organ or organs agglutinins originate. Achard and Bensaude, Arloing, Widal, and Sicard endeavoured to ascertain whether, before passing into the blood, agglutinins were formed in the inflammatory fluid found at the point where a given microbe had been inoculated. Their conclusions were either negative or uncertain. Achard found the agglutinating power of pleuritic serous fluid sometimes greater, occasionally smaller, and at times equal, to that of the serum. Paul Courmont thought that occasionally agglutinins might be formed even in serous membranes. Pfeiffer and Marx, however, were the first to seek systematically for the place of formation of agglutinins. They injected cholera vibrios into young rabbits, killed the animals at varying times after inoculation, and observed that agglutinins were formed early in the lymphoid organs, the medulla of the bones, the lymphatic glands, and more especially in the spleen. Nevertheless, this fact was not sufficient to warrant them in drawing definitive conclusions.

Later, van Emden studied the distribution of the agglutinins of the bacillus *aërogenes* in the animal organism. According to this author, agglutinins were formed in the spleen, in the medulla of bone (which is able to compensate the spleen when that organ is removed), in the lymphatic glands (which, indeed, were sometimes richer in agglutinins than the spleen), and in the liver. But these organs contained more agglutinins than the blood only very soon after the injection of specific microbes. Later on, the blood was richer in agglutinins than these organs. These results were not confirmed by Rath who, however, used the bacillus *typhosus* for his experiments.

In the course of his researches on the resorption of cells Metchnikoff observed several times that agglutinins appeared first in the peritoneal exudations, and in the blood several days afterwards. Later on, the peritoneal liquid became less active than the serum. Metchnikoff was inclined to attribute a preponderating share in the production of agglutinins to the cells found in the peritoneal inflammatory fluid.

Deutsch observed in his interesting researches on anti-typhoid bodies that in immunized animals the liver, the kidneys, and the suprarenal bodies contain traces of agglutinins merely; whereas varying quantities of agglutinins could be obtained from the lymphoid organs—spleen, medulla of bones, lymphatic glands—though these organs