Observations and experiments with the microscope, on the chemical effects of chloral hydrate, chloroform, prussic acid, and other agents, on the blood / by Thomas Shearman Ralph.

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Observations and Experiments with the Microscope, on the Chemical Effects of Chloral Hydrate, Chloroform, Prussic Acid, and other agents, on the Blood.

By Thomas Shearman Ralph, M.R.C.S., Eng., &c.

[Read before the Medical Society of Victoria, 1st February, 1871.]

Mr. President and gentlemen,—On a former occasion, now five years ago, I had the honor of reading before the Medical Society of Victoria, a paper entitled, "Observations and Experiments with the Microscope, on the effects of Prussic Acid on the Animal Economy," in which I pointed out the specific or chemical action of that agent on the blood, viz: that the iron was laid hold of by the cyanogen, and the result was the formation of prussian blue, or some cyanic compound of iron. Accompanying this remarkable change, was another, which I also pointed out. That certain oval bodies, closely resembling starch grains, were formed; these bodies turning blue under the action of iodine, and polarizing, were seen to form in the field of the microscope.

After my communication on the effects of Prussic Acid, I investigated the action of another chemical agent, which exhibits decided effects on the corpuscles of the blood, when applied to them out of the body; namely, Ammonio-Sulphate of Copper. When blood is allowed to flow into a solution of this compound, it is found that the contained matter of the red corpuscles cannot pass out: for when blood is drawn and placed in a thin film on glass, and examined under the microscope, it is found that the major part of the corpuscles gives up the contained matter, and the empty cell walls or coverings remain behind. This is well seen on applying a solution of magenta to blood under the microscope; the field becomes occupied by a vast amount of granular matter, coloured red by the dye; while the cell walls or envelopes lie in abundance uncoloured; or at the most presenting to the eye of the observer, the red molecule first pointed out by Dr. Wm. Roberts, of Manchester, in 1863, and also brought further into notice by Professor Halford, in 1864, before this Society.* My experiments with this agent, Ammonio-Sulphate of Copper, go to show, that while the corpuscles

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* Australian Medical Journal, vol. ix., 1864.

are so acted on, that they cannot pass out their contents, yet when magenta is applied, this dye can pass in and colour them ; and this colouration shows by its tint the degree of emptiness or fulness of each corpuscle ; proving, that at the moment when the cupreous solution was added to the fresh-flowing blood, the corpuscles were in different conditions ; some perfectly full, while others were partially empty. By means of water, the cupreous compound can be washed away, and then these same corpuscles are able to part with their contents, as they do under ordinary circumstances.*

Subsequently, I offered some observations on the action of Snake poison on the blood ; *i. e.*, that it could be compared to that brought about by Prussic Acid ;—that this agent, while it attacks the iron in the blood, yet sets up a further action ;—that of causing the newly formed red corpuscles to retrograde, as it were, to the condition of the white.†

Here are three important chemical agents, which have been applied to the blood, in order to elicit information regarding either its structure or its chemical character, namely :—

Magenta ; which was first taken in hand, and which attacks the nuclei of the white corpuscles and colours them ; also, the granular matter exuded from the red.

Ammonio-Sulphate of Copper ; which prevents the egress of the solid portion of the red corpuscles; while, at the same time, magenta can pass in and colour them effectually.

Prussic Acid ; which lays hold of the iron in the blood, withdrawing it from some organic state of combination ; giving rise also to the formation of corpora amylacea or starchy bodies, by some further change effected on the blood constituents.

I feel it is necessary thus to enter upon a resumé of what has been done, in order that what follows may be rendered more clear, and that the minds of those to whom I address myself may be satisfied that all the following observations and experiments have proceeded gradatim, and owe their origin, and are connected, with my former labours in this field of observation.

In bringing forward the present communication, I feel more and more satisfied of the importance of that mode of investigation which I have employed ; that it is one which opens another avenue to the study of physiology, as well as leading us to the ultimate, or chemical action of agents on the animal economy.

Experience and observation, based on the separate and combined action of the above mentioned agents, have satisfied me that some reliable chemical effects may be traced out regarding other agents, whose action on this portion of the animal economy are as yet unknown.

The difficulty hitherto has been to find an agent, the effects of which either exceed or distinctly differ from those of any substances hitherto recognised ; while, at the same time, the nature and probable

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* Id. : vol. xi., 1866.
† Id. : vol. xii., 1867.

action of the new agent should be such as we can trace out without encountering serious difficulty as to its interpretation.

I now proceed to the demonstration of some chemical changes in the blood, produced by means of different agents; the effects of which have been hitherto entirely unknown, and which will prove suggestive to the mind of the medical practitioner as soon as he shall have presented to him a further series of experiments carried out after the mode of investigation which I have endeavoured to follow, namely: the general action of a chemical substance on the blood withdrawn from the body, and traced out by the microscope; and also the investigation of the action of the same agent on the blood, after it has circulated through the animal economy, having been thereby subjected to the continuous action of air during its passage through the lungs. In the former instance, we obtain a general kind of action on the blood; in the latter, more positive or distinct effects are presented to our notice, as I shall endeavour to point out by and bye.

The examination of the blood drawn from the circulation and subjected to the action of a chemical agent, does not suffice to show us all that may be produced on it by that agent, and we need, if it is possible, to ascertain and compare its effects after it has circulated in the system. This is true, however, only to a certain extent, for we know that magenta has a remarkable effect on the blood when added to it; but we find no trace of its effects on that fluid, when we inject it under the skin, or pass it into the stomach with a view to its absorption and subsequent action on the blood, as the experiments of Professor Halford go to show.

I now pass on to the examinationI have made of the action of one important chemical agent which has only lately been brought before the notice of the medical profession, both here and at home, or rather in Europe. I mean Chloral; or as its more chemical name is supposed to be "Trichloric Aldehyde." This substance is now in use as an Hydrate, and its action has been stated to be somewhat like Chloroform. When an alkaline solution is added to it, Chloroform is set free; hence its proposer, Liebreich, suggested its use: that meeting with alkaline elements in the blood, it might become decomcomposed into Chloroform This theory, which is a very taking one, was no doubt the cause of the experimental use of this substance; the general experience, however, of the profession is against the idea that it acts as an anæsthetic, but only as a true hypnotic.

I feel inclined to the opinion that though it appears almost certain that Chloroform is eliminated in the blood by its decomposition; yet that the action of that agent is considerably modified by the attendant chemical change which necessarily accompanies the decomposition of this agent in the blood. If it is correct that the Hydrate of Chloral is decomposed by the alkaline state of the blood; then it follows as certainly that the resulting compounds must be Chloroform and a formate of some alkali. And if we regard the presence of alkalinity to be normal in the blood, then we obtain not only the chemical or physiological action of Chloroform, but we have also to

consider what may be the physiological effect of the formate of an alkali, whether of Ammonia or Potassa, and on such grounds we may fairly deduce that the physiological effects of Chloral on the animal economy must be somewhat different to that of Chloroform by itself; hence, perhaps hypnotism, in place of anæsthesia.*

At the risk of being tedious, I now approach the demonstration I have proposed to make, by reference to the line of my own experience in this matter; and I extract the following from my microscopical note book. "August 22nd, 1870.—Hydrate of Chloral is a remarkable chemical substance, producing a singular effect on the blood when applied directly to it. A small portion placed on a glass slide and slightly moistened, and then fluid blood added; about one-third of the corpuscles appear to corrugate their solid contents, which then take colour from Magenta."

This was the first fact which attracted my notice; the red corpuscles of the blood when acted upon by Magenta, under ordinary circumstances pass out or give up their contents, which then become coloured by the dye: in this instance, the dye penetrated the corpuscles, and coloured the material within them.

This effect was sufficient to indicate to my mind the remarkable chemical action of Hydrate of Chloral, and having made a note of it, I waited until this agent was within my reach for further experiment; for when I made this observation I could only obtain a few grains of it for experimental purposes.

In the following October I had occasion to administer it in small doses with the view of relieving pain; this enabled me to examine the condition of the blood. The blood, drawn two or three hours after its exhibition, presented a remarkable appearance. In several parts of the field of the microscope, besides *garnet-coloured amorphous particles*, a number of *red coloured globules* (double the diameter of white corpuscles, and many smaller) were seen; some of these were dark red. This was experiment the first.

Experiment 2.—Hydrate of Chloral was exhibited by the stomach to a rabbit: within an hour red masses were seen in the blood, also the presence of starchy bodies were noticed.

Experiment 3.—A frog was subcutaneously injected with Hydrate of Chloral, with the same results.

Experiment 4.—Frog immersed in a four-grain solution of the

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* The decomposition of Chloral Hydrate by Ammonia is curious to witness, when carried out in the following manner; a solution of the Hydrate should be placed in a narrow tube, about seven or eight inches long, and Ammonia added, and the mixture shaken and slightly warmed, when a white cloudiness will make its appearance, and bubbles of gas rise to the surface. If now a little superstratum of water is added, and not allowed to mix with the contents of the tube, the bubbles of gas will be seen passing through this stratum of water, and with a pocket lens the decomposition will be well seen. So soon as a bubble reaches the surface and disappears, from that point there descends an oily looking fluid (Chloroform); but before this reaches the cloudy portion, an amorphous or semi-crystalline material is formed—formate of Ammonia;—what the gaseous portion is, I have not ascertained.

Hydrate for some hours, when it was found hypnotic. Blood, nuclei of corpuscles appeared greenish, red particles also seen.

Experiment 5.—Frog killed by Hydrate of Chloral, after some hours of sleep. Blood from heart decidedly tinged redder than usual; some corpuscles presented reddish dots on their surface; red coloured masses were noticed all through the blood, as seen before.

Experiment 6.—On self. Three grains of Hydrate of Chloral were taken about two hours after a meal; the blood was examined every quarter of an hour; at the end of an hour it exhibited decided reaction; blue as well as red particles were seen. When the blood had dried on the glass slide and under the covering glass (which was about two hours after), some spaces, where coagulation had taken place, were filled with fluid presenting either a blueish or reddish tint. The urine also exhibited some dark coloured and reddish particles.

Experiment 7 and 8.—Two rats were killed, one by Chloroform, the other by Hydrate of Chloral, injected subcutaneously. This last took a grain and a half before deep sleep was induced. Blood exhibited ruby red particles; a few blueish; also starchy bodies in abundance. The urine also showed the same.

The chloroformed rat.—Urine with abundance of starchy bodies, and some blue coloured particles; blood from lungs—plasma reddish; few starchy bodies; some blue particles; *scarcely* any reddish.

Experiment 9.—Rat injected with Hydrate of Chloral. Deep hypnotism; blood gave the same results; starchy bodies and red coloured masses. Ammonia inhaled appeared to increase the production of the red matter. A solution of Ammonia injected under the skin appeared to give rise to bright red smears, or fluidity, between the corpuscles. The blood, under the action of Ammonia, in both forms of exhibition, seemed to have assumed a redder tint than usual.

Experiment 10.—A newly-born rat was placed in a solution of Hydrate of Chloral. After some hours the blood exhibited redness in the liquor sanguinis; also some fine red particles and red patches.

Experiment 11.—Hydrate of Chloral was evaporated from a slide on to fresh blood held over it; bright red coloured particles were formed in it.

Experiment 12.—Blood exposed to the vapour of Chloroform gave some evidence of red coloured matter. It appeared to me, at this point of my experiments, that the chemical action of Hydrate of Chloral on the blood was mainly due to Formyl, or Formic Acid, produced by its decomposition. When more Ammonia was introduced into the experiment, a larger production of the red material resulted.

Experiment 13.—Formic Acid (obtained from ants) added to blood gave rise to the formation of dark red globules and particles.

Experiment 14.—Lactic Acid added to blood yielded red particles; these appeared to increase on the addition of Prussic Acid; the fluid or plasma appeared redder.

Experiment 15.—Blood, with Prussic Acid added and then Oxalic Acid, yielded red-coloured particles.

Experiment 16.—Blood exposed to vapour of Hydrate of Chloral gave red particles as before; these lost their colour on addition of Oxalic Acid.

Experiment 17.—Prussic Acid and Ammonia were mixed together on a slide, and fresh blood added; red particles made their appearance; no starchy bodies; blood corpuscles and plasma redder than usual.

Experiment 18.—Blood, exposed to Ammonia vapour, became slightly reddened; Prussic Acid added in fluid form; blood became decidedly redder; red particles and red plasma resulted.

As far as these experiments had gone, I considered it reasonable to conclude that the decomposition of Hydrate of Chloral in the blood gave rise to the liberation of Formyl, or else Formate of Ammonia. But what becomes of it? Is it likely to remain in a free and uncombined state? or rather does it not combine with that important element in the blood—iron, producing a formate of iron; or perhaps Ammonia-formate of iron?

These decompositions in a highly complex material, as blood, are most difficult of explanation; and it is here I feel we must advance with caution.

The next point to which I directed my attention was to ascertain the action of Hydrate of Chloral upon a salt of iron; and the following experiments appear to me to support the view I have just expressed.

Experiment.—Chloral dissolved in a little water with Ammonia added, was followed by the decomposition of the former; a crystal of Sulphate of Iron was added, and the effect watched under the microscope; red-coloured particles and amorphous masses of different depths of tint, closely resembling those seen in the blood in the forementioned experiments, made their appearance.

A solution of Ammonio Citrate of Iron also gives similar results, and somewhat similar also is the action of Prussic Acid and Ammonia conjointly acting on a salt of iron.

Again, being aware from experiments performed in times past, that the presence of iron in vegetable tissues was demonstrable by means of Prussic Acid and Prussic Salts, I proceeded to make the following experiment, which, if it does not convince by its single testimony, yet is to my mind highly satisfactory; it is also one of the most remarkable of the kind which I can adduce, in relation to chemical action on vegetable tissues as revealed by the microscope; when once witnessed it can never be forgotten.

Experiment.—A section of a tender vine was made and placed on a glass slide with water, and Chloral Hydrate added; a reddish tint pervaded some of the cells; but when Ammonia and Chloral were added together, the tissue became tinged with a dirty red.

Again: Prussic Acid and Ammonia combined were added to another vine section, and produced a most beautiful and striking reaction. The woody ducts were seen filling up with bright red

fluid. In both these instances I have no doubt Formyl, or Formic Acid, attacks the iron combined with the fluids or tissue of the plant.

I can adduce a number of similar experiments, carried out with the same chemical agents, which more or less yield evidence of a similar kind of reaction; but this would prove tedious and superfluous.

I now proceed to sum up my experiments. Hydrate of Chloral administered by the stomach or subcutaneously injected gives rise to the production of bright red or dark red particles, masses, or globules, in the blood. Starchy bodies are also met with accompanying these charges. The urine also exhibits these bodies. The same results follow, when vapour of Hydrate of Chloral is applied to fresh drawn blood.

Ammonia, administered by the lungs, or subcutaneously injected during the action of Hydrate of Chloral on the animal economy, appears to heighten these effects.

Formic Acid added to fresh blood also causes the production of dark red globules and particles.

Lactic Acid conjoined with Prussic Acid does the same.

Prussic Acid and Ammonia conjoined yields the same results.

The action of Hydrate of Chloral, while decomposing under Ammonia on a salt of iron, presents changes which to my mind are identical.

The chemical effects of Hydrate of Chloral and Ammonia, of Prussic Acid and Ammonia, on some vegetable tissues, appear to be much the same in character as those produced in the blood, minus of course, the solid albuminous matter. All these results I refer to the action of Formyl or Ammonio-formate on the iron in the blood, or in the vegetable tissues.

The decomposition of Lactic Acid with Prussic Acid can supply chemically the elements necessary for the production of Formyl or Formate of Ammonia; as also Prussic Acid and Ammonia.

There are one or two more experiments to which I must refer, *i.e.*, the chloroformed rat, in which the blood was noticed to be reddish, but scarcely any red matter was seen. It would appear the chemical condition of the blood is not capable of readily decomposing Chloroform; such also is the case, I believe, with Hydrate of Chloral applied to the blood out of the body; but when the vapour of Chloroform, or the Hydrate of Chloral is applied, then the red particles make their appearance.

Here is another remarkable occurrence which receives its solution from the forementioned experiments. Some blood was accidentally examined after wine (Reisling) had been taken; this was with the view of exhibiting the action of Prussic Acid on iron in the blood; but it was noticed little or no reaction could be found after its application; but a good many red coloured globules and particles were seen, just as if Chloral had been taken. In consequence of this, a small drop of the wine was added to a little blood fresh drawn; the changes seen under the microscope were most remark-

able. Abundance of globules of a dark red or brown colour made their appearance, as also red amorphous masses or particles. Gas also was given off in the neighbourhood of the globules. Some of these bubbles contained a blueish fluid; the nuclei of the white corpuscles were blueish.

The experience I have already gained in carrying out these experiments leads me to see that the condition of the blood recognisably varies from day to day, from the effects of food, &c.; for the varying degrees of success which have attended a number of experiments performed with the same chemical agent, on the same individual, point to the great probability of the variable condition of the blood, when that individual has been the subject of variety in diet, or degrees of fatigue of mind or body.

Another consideration which presents itself to my mind is, that just as we now test the condition of the urine in order to ascertain what is being eliminated from the body of a patient, so will the physician find it useful occasionally to test, by means of reagents, the condition of the blood of his patient, in order to verify the character of some obscure symptoms. Even at this period of my experience, I have reason to believe it is possible by means of agents previously administered, to prolong the hypnotic action of Chloral, or to prevent or modify it in a great degree.

Thus, I believe I have at least been able to give demonstration to the theory of Liebreich, who, by his chemical knowledge, has enabled the medical practitioner to employ a remarkable agent, one which promises to be a sister companion to Chloroform in alleviating the ills to which flesh is heir. I hope I may be fortunate enough to arouse the attention of my professional brethren to the investigation of the chemical action of remedies on the blood, and thereby, perhaps, lead on to a more rational and satisfactory mode of treating some diseases, which in time to come, I believe, will be attacked directly through the blood itself.

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