"On the Part played by Benzene in Poisoning by Coal Gas." By R. STAEHELIN, M.D., Senior Assistant in the Medical Clinic at Basle. Communicated by Professor E. H. STARLING, F.R.S. Received December 11, 1903,—Read January 28, 1904.

(From the Physiological Laboratory, University College.)

In a recent paper Vahlen* has maintained that a difference exists between the poisonous action of coal gas and of carbon monoxide, and Kunkel† has also drawn attention to a similar difference in the case of frogs. In the course of a research which I was undertaking in University College for other purposes, at the suggestion of Professor Starling, I have come across facts which may serve to explain the difference noted by these observers.

My first object was to investigate the effect of deprivation of oxygen on the fatigue curve of muscles. To this end, a muscle was hung up in a closed chamber and the atmospheric air driven out by a stream of some other gas. When coal gas was used for this purpose, it was noticed that the muscle rapidly went into *rigor mortis*, whereas, in nitrogen, it remained excitable for many hours. I set myself, therefore, to find out which constituent of the coal gas was responsible for this poisonous effect.

The experiments were carried out on the frog's sartorius, since the relatively large surface of this muscle renders it particularly accessible to gaseous poisons. The muscle was hung up in a glass tube, closed above and below by rubber corks. To the upper cork a hook was fastened on which was hung the bony insertion of the sartorius. Through the lower cork a glass tube passed. The movements of the muscle were transmitted to a recording lever by means of a steel needle hooked into the lower end of the muscle and passing through the glass tube. The lever was weighted near its axle, and was afterloaded, and its movements were recorded on a slowly rotating drum, on which the time was also marked by means of a signal. The closed tube was provided with two small lateral openings by which any gas desired could be passed through it, the opening through which the needle passed to the lever being made sufficiently air-tight by means of a small plug of vaseline. In every experiment two similar

* Ferchland und Vahlen, "Ueber Verschiedenheit von Leuchtgas- und Kohlenoxydvergiftung," 'Archiv für exper. Pathologie und Pharmakologie,' 48, p. 106; Vahlen, "Ueber Leuchtgasvergiftung," 'Archiv für experimentelle Pathologie und Pharmakologie,' 49, p. 245.

† Kunkel, "Ueber Verschiedenheit von Leuchtgas- und Kohlenoxydvergiftung," 'Sitzungsberichte der Physikalisch-medizinischen Gesellschaft zu Würzburg,' 1902, Nr. 4, 5, p. 61.

apparatus were employed, so that the influence of two gases could be compared at one and the same time on the two sartorii of the same frog.

When coal gas was led through the tube the muscle began to contract 1—7 minutes after the beginning of the passage of the gas, and in ½—1 hour the muscle was maximally contracted and had an opaque appearance. The rapidity with which the contraction came on was proportional to the rapidity with which the gas was passed through the chamber, and varied with the temperature, warmth quickening and cold slowing the process. Once the muscle was fully contracted no recovery took place. This phenomenon could not be due to the CO in the coal gas, since, on passing pure CO through the muscle chamber, the muscle remained excitable as long as in nitrogen, and the fatigue curve took the same course as in that gas. Among the remaining more important constituents of coal gas which might produce this phenomenon, benzene merited first attention, since all aromatic bodies are more or less potent poisons. I therefore tried the effect of passing air through benzene into the muscle chamber. I found that in less than a minute the muscle began to contract, and the contraction reached its maximum height within a short time, the muscle becoming opaque and dead. The other sartorius of the same frog was placed at the same time in coal gas. It began to contract only after several minutes, and the contraction took half an hour to reach its maximum. Benzene vapour therefore showed itself much more poisonous for a muscle than coal gas, evidently on account of its greater concentration. According to Letheby, London coal gas contains only 3·8 per cent. of condensible hydrocarbons, of which benzene forms only a small proportion. I therefore, in another experiment, passed air into the muscle chamber, not through pure benzene, but through water which had been shaken up with benzene. In this case the contraction began 6 minutes after the beginning of the experiment and took 38 minutes to reach its maximum, while the control muscle in coal gas began to contract in 7 minutes and reached its maximum contraction in 40 minutes.

It seemed, therefore, highly probable that benzene was really the constituent of coal gas which was responsible for its toxic properties on muscle. If that were the case the passage of coal gas through oil, which absorbs benzene, ought to deprive it of its deleterious effects. This was found to be the case. Coal gas, passed through oil, showed no difference in its effects from pure CO or nitrogen.

In the same manner as benzene I investigated the effects of xylol and toluol. If the muscle was supplied with air blown through either of these fluids no poisonous effect was observed even when the fluids were warmed. The absence of effect in these two cases is perhaps to be ascribed to the lower vapour tension of these two substances. The

muscle was equally unaffected when supplied with air which had been passed over heated naphthaline.

I also investigated the effect of certain hydrocarbons of the fatty series, namely, methane, prepared by heating sodium acetate and sodium hydrate, acetylene produced by the action of water on calcium carbide, as well as the mixture of substances obtained by blowing air through petroleum ether. In none of these cases was any effect produced on the muscle. If, however, a trace of benzene was added to petroleum ether, *rigor mortis* of the muscle was almost immediately produced. In this case the volatility of the petroleum ether apparently aids the evolution of the benzene. On the other hand, the addition of small quantities of benzene to xylol does not impart poisonous qualities to the air bubbled through the mixture, the benzene being apparently held fast by the less volatile xylol.

It seemed, therefore, most probable that the specific poisonous effect of coal gas on frog's muscle was due to benzene only, and that it was to the presence of this substance in coal gas that the differences observed by Kunkel and Vahlen between the action of coal gas and carbon monoxide were to be referred. Vahlen states that warm-blooded animals and frogs die more rapidly in coal gas than would be expected from its percentage of CO, and also that frogs in coal gas present excitatory phenomena which are absent in pure CO. Although Kunkel denies the presence of any difference between the action of these two gases on warm-blooded animals, he also draws attention to the peculiar effects on frogs of coal gas, which are not produced by other gases free from oxygen, and describes them as "choreiform twitchings and spasms in the neck and legs." To decide this question the following experiments were carried out:—

I. Three frogs were placed in air-tight bell jars, through each of which coal gas was conducted at constant rate. The coal gas had to pass in each case through a wash bottle, which in A was water, which, of course, left the composition of the gas unchanged, in B oil, which would absorb the benzene. In front of C were two wash bottles, the first one containing oil, the second one benzene.

The passage of the gas through the three bell jars was begun at 11.20. At 11.25 all three frogs were restless. Frog C remained then still for a short time, and the breathing became irregular, and twitching occurred in the extremities and back. Movements were chiefly co-ordinated, though there were some twitchings of isolated muscles. After a little time the movements became shorter and less co-ordinated, the legs remained stretched out, and breathing ceased. Frog A betrayed phenomena similar to C, but the spasms were less evident, and came on more slowly. Frog B became quite quiet, the respiration becoming irregular and shallower.

At 11.40 B was sitting up in normal position, though the breathing was somewhat irregular, while A and C were lying on their bellies, with legs stretched out. At 11.45 all three frogs were taken out. C gave no signs of life, and in ten minutes was quite rigid; B still reacted slightly to stimulation, showed shallow respiratory movements, and gradually recovered, so that at 1.30 it was apparently normal. Frog A at first showed no response to stimulus, and no respiratory

movements. By 11.55 it had recovered sufficiently to show both these phenomena, and at 1.30 it was so far recovered that it could recover its position when turned on its back, and in a couple of hours later was apparently normal.

II. In a second experiment the arrangements were the same as in the first, except that in C the gas which had passed through the oil was allowed to pass through water saturated with benzene instead of through pure benzene. In this case the phenomena in A and C were practically identical, and were similar to those observed in A in the first experiment. We need not, therefore, give fuller details of this experiment.

We thus see that, when frogs are exposed to coal gas, motor phenomena are produced, which are absent if the coal gas be previously purified by passage through oil, and that these phenomena can be reproduced if the purified gas be made to take up benzene vapour. The poisonous properties of the gas can be increased by increasing the tension of the benzene vapour. We are, therefore, justified in concluding that the differences between the effects of CO and coal gas observed by Kunkel and Vahlen, depend on the presence in the latter of benzene. The slight motor excitation observed in frogs in coal gas, which had been purified by passage through oil, is exactly similar to that described by Kunkel, as the result of deprivation of oxygen, produced by placing the frogs in nitrogen or CO, as is shown by the following experiment:—

III. Two frogs were placed, one in a bell jar through which CO gas was led, the other in a similar jar through which coal gas was led after passing through oil. Both animals in a short time showed slight twitchings of isolated groups of muscles in the extremities and back, and occasional extensor movements of the hind limbs, which gradually diminished. No difference was observable between the two frogs. In three quarters of an hour they were taken out of the jars, and both recovered within a short time.

In order to be certain of the part played by benzene in coal gas poisoning, we must have some idea of the effect of pure benzene on the frogs. I have been unable to find any published experiments over the effects of inhalation of benzene on the frog. Beyer* states that xylol acts as a narcotic poison, like the other odorous substances investigated by him. I have, therefore, made some experiments on the influence of benzene vapour in the presence of oxygen on frogs.

IV. A frog was placed in a bell jar, in which a beaker of benzene was hung up. Eight minutes after the beginning of the experiment spasmodic movements and twitchings began in various parts of the body, accompanied by a considerable secretion of mucus. After a few minutes the spasms ceased, the frog lay still with extended limbs, and respiration, which at first was irregular, became gradually shallower and less frequent. Twenty minutes after the beginning of the experiment all respiratory and other movements had ceased. The frog was taken out of the jar, and recovered in a few hours.

* Beyer, "Narkotische Wirkungen von Riechstoffen und ihr Einfluss auf die motorischen Nerven des Frosches." 'Archiv für Anatomie und Physiologie, physiologische Abteilung,' 1902, p. 201.

In other experiments in which the frog was left longer exposed to the action of benzene vapour, *rigor mortis* came on first in the hinder and then in the fore extremities before the heart had ceased to beat. One peculiarity was observed with regard to the reflex irritability of the animals, under these conditions, which is worthy of notice. Very early in the experiment, at the very beginning of the spasmodic movements, the frog reacted very slightly and incompletely to changes in its position, that is, such as would be produced by holding the vessel in which they were placed in an oblique position or turning them on their sides or backs. On the other hand, the reactions to tactile stimuli of the skin were much more pronounced than usual, so that a tap on the one foot might evoke muscular contractions throughout the whole body. When the animals began to become rigid, stimulation of a toe of the rigid limb could evoke contractions of the limbs which had not yet become stiff. Thus, in all these experiments the first result of the poisoning was motor excitation, which showed itself at first by co-ordinated movements affecting large portions of the body, and later by twitchings of isolated groups of muscles. A little later the respiration became irregular and finally ceased. The higher reflexes, *e.g.*, the reaction to changes in position, were abolished, while the lower were increased. Finally, however, the paralysis became universal, so that also the spinal reflexes were abolished. This stage was followed by a rigidity of the muscles, and last of all the heart ceased to beat. The rapidity of onset of these phenomena is naturally dependent on temperature, being quicker the higher the external temperature. It is evident that we have, therefore, to deal with an action of the poison on the central nervous system. The general spasmodic movements are abolished by previous destruction of the brain and spinal cord; the twitchings of the muscles and the rigor of the extremities persist, however, in the complete absence of the central nervous system, and occur in a hind limb, the nerve of which has been divided, as rapidly as on the opposite side. The onset is not prevented by curarisation, and must, therefore, be ascribed to a direct action of the benzene on the muscles themselves, as has been described at the beginning of this paper.

In the poisoning by coal gas this rigor of the muscles was not observed either by myself or Vahlen and Kunkel, probably because the animals die of asphyxia before the small amounts of benzene present in the coal gas have time to bring about their direct effect upon the muscular tissue. As Experiment I shows, the increase in the percentage of benzene in the coal gas is followed by the onset of rigidity in the muscles.

Rather more difficult is the explanation of the increased reflex excitability in the later stages of intoxication. It may be that the poisonous effects are first confined to the higher centres. On the

other hand, the increased irritability of the muscles themselves is the chief factor in the spinal reflex hyper-excitability. In coal gas poison, when the lower centres are also paralysed by asphyxia, the reflex excitability disappears.

It is thus possible to refer the difference between intoxication by coal gas and that by CO entirely to the influence of benzene, which determines in its first stage vigorous excitatory motor phenomena. In warm-blooded animals the conditions are quite different. Lorraine Smith* found that an addition of 0·65 per cent. of benzene to air had no effects on a guinea-pig, and Santesson† did not succeed in producing either acute or chronic poisonous effects by administering benzene to a rabbit by inhalation. In man, too, cases of poisoning by benzene are few and far between. It is possible that the minute quantities, which are absorbed by the lungs, are rapidly oxidised and excreted as an aromatic sulphate. At any rate, in man, benzene plays no part in the poisonous effects of coal gas.

Summary of Results.

(1) Coal gas produces first excitation and then rigor of the isolated frog's muscle.

(2) Frogs exposed to coal gas show excitatory phenomena which are absent when the animal is placed in an atmosphere of CO or nitrogen.

(3) The specific effects of coal gas on frogs are determined by the presence of benzene in the gas, and can be produced by air containing the same percentage of benzene.

(4) There is no reason to suppose that the poisonous effect of coal gas on mammals is determined by anything except its content in CO.

* Lorrain Smith, "The Poisonous Action of Coal-Gas and Carburetted Water-Gas." 'Report of the Water-Gas Committee,' presented to both Houses of Parliament by command of Her Majesty, 1899, Appendix VII, p. 127.

† Santesson, "Ueber chronische Vergiftungen mit Steinkohlenbenzin," 'Archiv für Hygiene,' 31, p. 336.