

# THE TOXICOLOGY OF NICKEL CARBONYL.

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(From the Lister Institute of Preventive Medicine.)

(Four Figures.)

## PART I.

### *Introduction.*

NICKEL carbonyl was discovered by Mond, Langer, and Quincke (1890). On passing a current of carbon monoxide over finely divided (pyrophoric) metallic nickel, they discovered that a gaseous compound of nickel and carbon monoxide was formed, having the composition of  $\text{Ni}(\text{CO})_4$ .

When the gas is heated to  $150^\circ \text{C}$ . it is decomposed into its constituents, and metallic nickel is deposited. The volume of liberated carbon monoxide after dissociation is four times that of the undissociated nickel carbonyl vapour. The gas can be condensed to a mobile liquid, if cooled in a refrigerating mixture. Fluid nickel carbonyl boils at  $43^\circ \text{C}$ . and solidifies into needle-shaped crystals at  $-25^\circ \text{C}$ . The vapour density at  $50^\circ \text{C}$ . is 8.69.

Further investigations on this compound were conducted by Mond and Nasini (1891), Berthelot (1901), Mittasch (1902) and Dewar and Jones (1903).

Nickel carbonyl is a clear, pale straw-coloured liquid, volatilising at room temperature. It has a peculiar soot-like smell, which can be detected in extremely small quantities (about 1 volume in 2,000,000), while the Bunsen flame becomes luminous when nickel carbonyl is present in air to the extent of 1 volume in 400,000. The liquid is soluble in alcohol, benzene and chloroform. It is not acted upon by alkalis nor by weak acids. Strong hydrochloric acid does not decompose it, but nitric acid and aqua regia do so easily.

Cobalt does not form a carbonyl, but iron does. Liquid iron carbonyl ( $\text{Fe}(\text{CO})_5$ ), when acted on by light, gives off one molecule of carbon monoxide, and a solid diferro-nonacarbonyl is formed, having the composition  $\text{Fe}_2(\text{CO})_9$ . Dewar and Jones (1906) also describe a tetracarbonyl of iron, which occurs in the form of short, dark green, lustrous prisms. It is almost insoluble and is not volatile. The pentacarbonyl is volatile at ordinary temperatures, but not to such an extent as the tetracarbonyl of nickel. It is made by passing carbon monoxide over reduced pyrophoric iron.

The discovery of nickel carbonyl was put to practical use by L. Mond for the manufacture of pure nickel. At first, it was not thought that the compound was more dangerous than the amount of carbon monoxide contained would suggest, and it appears that very little precaution was taken by the early investigators to avoid inhaling the vapour; fortunately without evil results.

However, when the substance was produced on a large scale, the accidental escape of the gas led to some unfortunate accidents, some four of which proved fatal.

The suggestion to investigate the toxicology of nickel carbonyl I owe to Dr Mond, who has placed a supply of nickel carbonyl and iron carbonyl at my disposal, and who has done much to render the work less difficult. Much assistance has been derived from the advice of his chemists, Drs Shields and Hirtz and also from Dr Langer, the Managing Director of the Mond Nickel Company's Works at Clydach, to all of whom I wish to express my thanks. Through the courtesy of Dr Mond I have been able to observe some mild cases of poisoning in man. It is therefore a great pleasure to me to express my thanks to Dr Mond in this place.

I wish also to express my grateful indebtedness to Dr Charles Martin, the Director of the Lister Institute of Preventive Medicine, for the very valuable assistance which he has given me, both in suggesting methods to overcome difficulties and also in criticising results; and to Dr Harden, whose advice on chemical matters has been a great help to me.

The symptoms of nickel carbonyl poisoning in man were as follows: immediately after having been exposed to air containing plant-gas, there was giddiness, and at times dyspnoea and vomiting. These symptoms passed off rapidly, as soon as the patients were brought into the fresh air. After from 12 to 36 hours, the dyspnoea returned, cyanosis appeared and the temperature began to be raised. Cough with more or less blood-stained sputum occurred on the second day or later.

The pulse rate became increased, but not in proportion to the respiratory rate. The heart remained normal. Delirium of varying types frequently occurred, and a variety of other signs of disturbance of the central nervous system was noted. Death took place in the fatal cases between the 4th and 11th days. The chief changes found post mortem were haemorrhages in the lungs, oedema of the lungs, haemorrhages in the white matter of the brain (in one case this was very extensive), while some doubt exists as to whether any blood changes were present.

At present it is not possible to state what the lethal dose for man may be.

In animals poisoned with nickel carbonyl vapour, analogous symptoms and post mortem changes have been observed. It is proposed to deal with these in detail in a subsequent communication. The post mortem changes in animals (rabbits, cats and dogs) consist of haemorrhages in the upper air passages, haemorrhages, oedema and compensatory emphysema of the lungs, haemorrhages in the adrenals and in the central nervous system. Haemorrhages occur less commonly in the kidneys, still less commonly in the spleen and very rarely in the liver.

The inhalation of air containing 0.018 volume % of nickel carbonyl vapour for 65 minutes is just sufficient to kill rabbits; 0.04 volume % has to be inhaled for 90 minutes by cats to produce death, while dogs die if exposed to 0.036 volume % for 90 minutes. No symptoms appear for the first 12 to 24 hours after the inhalation.

A few observers have studied the poisonous effects of nickel carbonyl. Henriot and Richet (1891) came to the conclusion that nickel carbonyl is taken up by the blood and is slowly split up. They found carbon monoxide haemoglobin. Langlois (1891) believes that nickel carbonyl replaces the oxygen in the blood. Vahlen (1891) discusses the conclusions of these authors and comes to the conclusion that death is due to nickel carbonyl as such and not to carbon monoxide. McKendrick and Snodgrass (1891) ascribe the toxic action to nickel, on the basis of a small number of experiments, chiefly dealing with enormously excessive doses. A number of earlier workers have dealt with the toxicology of nickel salts, among whom, Anderson Stuart (1884), Ross (1887), Riche (1888), Laborde and Riche (1888) and Rohde (1889) may be mentioned.

*The Toxic Agent in Nickel Carbonyl poisoning.*

In considering what the toxic agent in nickel carbonyl poisoning may be, one has to deal with three possibilities:

1. That the carbon monoxide of the compound is wholly or partly responsible for the symptoms;
2. that nickel carbonyl is absorbed as such and acts toxically;
3. that the nickel of the compound produces the symptoms.

A modification of these may be considered, viz. that nickel carbonyl is absorbed as such and that the nickel being set free later, acts toxically in the tissues.

1. *Consideration of what part in the Toxicology may be played by Carbon Monoxide.*

When nickel carbonyl is dissociated, the carbon monoxide liberated occupies four times the volume of the original vapour. Shields has pointed out that if nickel carbonyl is shaken up with blood, the haemoglobin acquires the colour characteristics of carbon monoxide haemoglobin. In my poisoning experiments, when the minimum doses of vapour were used, the blood contained only a small amount of carbon monoxide, which seldom exceeded 5 %.

In order to show that carbon monoxide is not the toxic agent, the following facts can be brought forward:

1. In fatal poisoning from minimal doses the actual quantity employed is insufficient when combined with the haemoglobin to produce more than 5 % of saturation with carbon monoxide, and this amount is harmless.

2. The blood of an animal saturated to the extent of 32 % with carbon monoxide loses carbon monoxide during subsequent poisoning by nickel carbonyl.

3. Iron carbonyl is less toxic than nickel carbonyl, although it contains more carbon monoxide.

Ad. 1. It has been found in a large number of experiments, that the inhalation of 0.018 volume % of nickel carbonyl vapour in air for 65 minutes is sufficient to kill rabbits; 0.04 volume % for 90 minutes is sufficient to kill cats, and 0.036 volume % for 90 minutes to kill dogs. It cannot be supposed that 0.072 volume %, 0.16 volume % and 0.144 volume % of carbon monoxide in air when inhaled by rabbits, cats and dogs for 65, 90 and 90 minutes respectively would produce

fatal results. These figures represent the amount of carbon monoxide liberated, if all the nickel carbonyl were dissociated. According to Gruber (1883) 0.07 to 0.08 volume % of carbon monoxide even when inspired for days does not kill rabbits. Gréhant (quoted by Sachs, 1900) found that dogs die when exposed to 0.4 to 0.5 volume % for from  $\frac{3}{4}$  to  $1\frac{3}{4}$  hours.

During fatal poisoning in rabbits, samples of blood were taken and examined spectroscopically and by Haldane's carmine method (1895) for carbon monoxide haemoglobin. If carbon monoxide had been the toxic agent, one would have expected to find at least 50% of the haemoglobin saturated with carbon monoxide at the end of the inhalation period.

Ad. 2. Rabbits were exposed to carbon monoxide gas until the haemoglobin of the blood showed from 25 to 32% saturation. 30% of the haemoglobin of the first rabbit was saturated with carbon monoxide; the rabbit was then allowed to breathe air containing 0.0188 volume % of nickel carbonyl for 65 minutes, and died without exhibiting any additional symptoms. 25% of the haemoglobin of the second rabbit was saturated with carbon monoxide, and this rabbit was exposed to the same dose of nickel carbonyl. It too lived as long as the control and did not show any additional symptoms. 28.5% of the haemoglobin of the third rabbit was saturated with carbon monoxide and then the rabbit was exposed to 0.019 volume % of nickel carbonyl for 65 minutes. At the end of the inhalation the carbon monoxide haemoglobin had decreased to 25.7% of the total. Two other animals after inhaling sufficient carbon monoxide to saturate 25 and 32% of the haemoglobin of their blood respectively, recovered after severe illness on being poisoned by a dose of nickel carbonyl which killed 83.1% of rabbits subjected to it.

The toxic effect of nickel carbonyl is therefore not increased by a preliminary inhalation of carbon monoxide, provided that not more than 32% of the haemoglobin is saturated. The amount of carbon monoxide combined with the haemoglobin may actually diminish during the inhalation of nickel carbonyl.

Ad. 3. Nickel carbonyl contains less carbon monoxide than iron carbonyl. As has been stated, 0.018 volume % inhaled for 65 minutes of nickel carbonyl vapour in air is sufficient to kill rabbits. 0.025 volume % of iron carbonyl vapour is required to be inhaled for one hour to produce the same effects.

It will be shown subsequently that the course of the symptoms and

the death in nickel carbonyl poisoning is not like that of carbon monoxide poisoning.

*Conclusion.*

The foregoing facts prove that the toxic effect of nickel carbonyl is not produced by its carbon monoxide content. They also show that when the quantity of the nickel carbonyl vapour inhaled is only just sufficient to kill, the total available amount of carbon monoxide is insufficient to influence the course of poisoning.

2. *Consideration of what part in the Toxicology may be played by Nickel Carbonyl as such.*

In order to attack the problem on safe grounds, it was found necessary to ascertain how nickel carbonyl behaves when it comes into contact with the fluids and tissues of the body. This study involved the investigation of the following :

- (1) Under what conditions nickel carbonyl is dissociated.
- (2) What the solubility of nickel carbonyl in water at various temperatures and pressures is.
- (3) What the solubility in blood and in serum is.
- (4) Whether any compound of nickel carbonyl or nickel is formed in the blood, and
- (5) What becomes of the nickel in the body after any dissociation which may take place, has occurred.

*Vapour Tension of Nickel Carbonyl.*

The vapour tension of nickel carbonyl at temperatures between  $-9^{\circ}$  and  $+39^{\circ}$  C. was determined by Mond and Nasini (1891), Dewar and Jones (1903) and Mittasch (1902). The curves of these observers showed moderate agreement. It was, however, deemed advisable to redetermine the vapour pressure of the particular samples used in this investigation. The following method was employed. A tube was cleaned and closed at one end and then carefully dried. It was then filled with dry mercury and boiled out. The tube inverted over a bath of mercury was jacketed with water and a thermometer was also introduced into the jacket. A small tube provided with two stopcocks and with the lower end curved upwards for a few millimetres was then completely filled with nickel carbonyl, care being taken to exclude air in the lower portion. The lower curved end was then inserted under

the open end of the barometer tube and the lower tap opened. The nickel carbonyl was driven out of the small apparatus by warming the tube with the hand. The temperature was rapidly lowered to about 3° C. and then slowly taken up to 35° C. and again depressed to about 5° C. The vapour pressure at different temperatures was as follows:

Up values		Down values	
2.85° C.	167.61 mm.	34.25° C.	547.8 mm.
7.1	194.61	26.3	410.97
11.8	230.56	22.45	349.97
18.9	300.5	15.35	273.51
26.4	403.47	9.9	218.66
34.85	564.3	6.4	190.66

Fig. 1 is the curve interpolated from these values. The points determined by Dewar and Jones are given for comparison. The values determined at the lower and higher temperatures practically coincide, while the values in the middle of the curve (between 19° and 24° C.) are slightly lower than those determined by Dewar and Jones. The curve interpolated from these observers' determinations would therefore lie slightly higher and would be flatter.

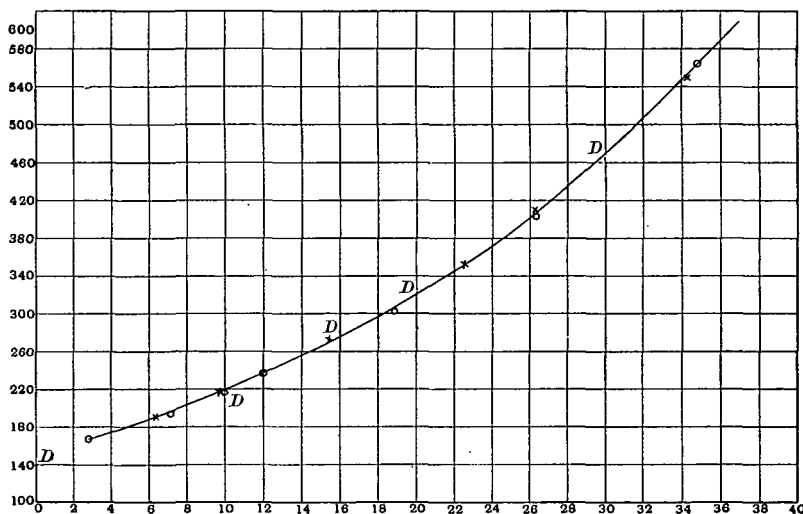


Fig. 1.

O = up values.

X = down values.

D = Dewar and Jones's points.

*Dissociation.*

When nickel carbonyl is kept in the presence of air and moisture, a deposit forms. This is due to the dissociation of the compound and the formation of a hydrate or carbonate of nickel.

In the burette containing nickel carbonyl, a deposit of hydrate or carbonate separates out after a few hours if any air is present. It is extremely difficult to keep liquid nickel carbonyl clear on account of this spontaneous dissociation, and the only safe way to avoid any deposit of hydrate is to fill the tube or burette with carbon monoxide after careful cleaning and drying.

Dewar (1903) noticed that when dissociation takes place out of contact with air and in the presence of an excess of carbon monoxide, recombination of part of the nickel with carbon monoxide occurs on cooling. In the vapour tension experiments, visible dissociation (*i.e.* black deposit on the tube) was seen at and above 22° C. Reversible action was noticed when a tube containing nickel carbonyl vapour and sealed at both ends, was heated at one spot. Under these circumstances a mirror of nickel is deposited on the glass, and at the periphery of the mirror a slight blackening is seen. This is most intense close to the edge of the mirror and diminishes imperceptibly further away from it. On cooling, some or all of this black deposit or even some of the mirror disappears again.

Some tubes of nickel carbonyl vapour with and without hydrogen were prepared and the dissociation watched. Evidence of reversible action was seen both in the tubes of pure nickel carbonyl and in those containing nickel carbonyl and hydrogen.

Slight dissociation was seen when tubes of nickel carbonyl vapour were kept in the water bath at 18° C. for several days. Dissociation takes place though slowly at lower temperatures. At room temperature (about 16° C.) a slight amount of black deposit was seen after 20 days in a flask containing water saturated with nickel carbonyl free from oxygen. At 35° C. nickel carbonyl dissociated fairly rapidly.

In an experiment dealing with the solubility of nickel carbonyl in water (to be described later) about 1.4 c.cm. of liquid nickel carbonyl was allowed to volatilise completely in an exhausted litre flask containing water at 35° C. Within 10 minutes a heavy black deposit of metallic nickel had separated out, and this increased steadily during the following two hours. The vapour pressure increased by 3 mm. in 10 minutes, and at the end of 20½ hours it had increased by 31 mm. from the carbon



monoxide set free. In a similar experiment, carried out at 23.4° C., enough nickel carbonyl was introduced into the flask to give a vapour pressure of 250 mm. After 20 minutes, the total pressure had increased by 11 mm.

A flask containing water, some mercury and an excess of nickel carbonyl, prepared anaerobically, was kept in the water bath at 29.7° C. After 14 days a black deposit had formed, in the fluid a thick layer was seen on the surface of the mercury and some black precipitate was adherent to the glass flask and capillary tubes.

When nickel carbonyl is evaporated into a large space containing air, a smokiness is seen, which may be due to floating particles of nickel hydrate or carbonate in extremely fine division.

#### *Solubility of Nickel Carbonyl in Water.*

The small solubility of nickel carbonyl and the fact that it dissociates so readily and also is apt to form an emulsion render it extremely difficult to determine the actual amount of nickel carbonyl taken up by water and aqueous fluids. The chief difficulties are due, firstly, to the fact that when nickel carbonyl in liquid condition is in excess, an emulsion is formed and the liquid does not settle readily, so that samples of the solution drawn off for examination frequently contain small amounts of liquid nickel carbonyl in suspension, and, secondly, that in dealing with partial pressures, dissociation takes place sufficiently extensively at temperatures above 10° C. to alter the pressure before equilibrium between the gases and the liquid has been obtained.

#### *Solubility at Constant Temperatures and Varying Pressures.*

The experiments were conducted at 9.8° C. The observations were made upon solutions which had been shaken with excess of liquid nickel carbonyl or had been saturated with gas at different partial pressures.

### METHODS.

#### 1. *Experiments with Liquid Nickel Carbonyl in excess.*

A small flask similar to those employed for washing gases, but with tubes of capillary bore, and provided with stopcocks, was filled with boiled water, after having been washed out with hydrogen. The water was boiled in a large flask and carried over into the smaller flask by its own steam pressure. During the cooling process the tap in connection

with the longer tube was left open and connected with a capillary tube filled with mercury and dipping into a mercury bath. After the temperature had fallen to room temperature, the mercury and some water were sucked out through the tap in the short tube into an exhausted flask. Nickel carbonyl was then placed into a burette with two stopcocks, between two seals of mercury, and this burette having been applied to the longer tube (all tubes being filled with mercury) both the tap of this tube and the lower one of the burette were opened, and nickel carbonyl allowed to flow into the flask together with the mercury. The tap was then closed, the flask well shaken and kept at a constant temperature in the water bath. The flask was kept in the water bath for about 24 hours during the earlier experiments, but when it was found that complete solution occurred within one hour, the sojourn was shortened to this period. The flask was shaken at frequent intervals while in the bath.

In order to determine the amount of nickel carbonyl dissolved, a sample of the water was sucked out into a second gas washing bottle, which had been well washed out with hydrogen and evacuated. Before taking this sample, the flask was inverted and allowed to stand so that any nickel carbonyl in suspension might settle. A few c.cm. of water were first drawn off through the longer tube, so that, should any globules of nickel carbonyl be sticking to the inside of the capillary tube, these would first be got rid of. The weight of the water drawn off for examination was determined by weighing the second gas washing bottle before and after taking the sample.

The bottle was placed in connection with a hydrogen apparatus and a combustion tube, having two asbestos plugs. The combustion tube rested on two Ramsay burners, being supported by copper troughs with lids. The tube was thoroughly heated, after which hydrogen was bubbled slowly through the fluid to drive off the dissolved nickel carbonyl. The hydrogen was burnt at the further end of the tube. The heat dissociated the nickel carbonyl and caused a mirror of nickel to form on the combustion tube close to the Ramsay burner, while some of the remaining nickel was deposited in the asbestos plugs.

The flame at the pointed end of the combustion tube indicated if the least trace of nickel carbonyl escaped dissociation. This never took place when the tube was properly heated, provided that the hydrogen was bubbled through the solution slowly at the beginning. Toward the end of this stage, the test employed was to allow the tube to cool, while the hydrogen was turned off, and then on lighting the

hydrogen again, one could immediately detect if any nickel carbonyl was still present. The flame test is so delicate (detecting 1 volume in 400,000) that no appreciable error occurred in carrying it out.

After the carbonyl had been completely driven off, the nickel mirror in the tube and the nickel in the asbestos plugs were dissolved in nitric acid. The solution was evaporated to dryness, and the residue treated with hydrochloric acid, again evaporated and dissolved in water. The fluid was made up to a given bulk and the nickel determined by the colorimetric method with dimethylglyoxime, described by A. Harden and myself (1906).

2. *Experiments in which Water was brought into equilibrium with the Gas at different Partial Pressures.*

A litre bolt-head flask with a special short neck was fitted with a rubber cork having five perforations. The perforations transmitted (1) a capillary tube dipping down to below the top level of the water, (2) a capillary tube dipping down to nearly the bottom of the flask, (3) a short tube to connect to the water pump, (4) a short tube to connect to the mercury gauge, and (5) a special burette for nickel carbonyl, with a three-way tap, through the one limb of which hydrogen could be let in.

The flask having been exhausted, the water was boiled (the mercury gauge being clamped off during this period to prevent distillation), any air given off was washed out by repeated displacement with hydrogen, and finally the flask was again exhausted. A measured quantity of nickel carbonyl was run into the flask from the burette and the flask was well shaken. A long tube containing mercury, which had been freed from water and air, was then attached to the capillary tube dipping to below the top level of the water. The sample was taken from the tube dipping nearly to the bottom of the flask and as it was being removed, mercury was let in, so that the upper level of the water was kept constant. The sample was dealt with as before.

One or two points in connection with the methods require especial attention. The hydrogen must be free from arsenic. Before displacing the nickel carbonyl, a blank test should be made each time to ensure that the rubber tubes connecting the parts of the apparatus (which should be as short as possible) have not retained any nickel carbonyl from a previous experiment dissolved in the rubber.

*Results.*

The following table gives the results obtained at 9.8° C. with varying pressures of nickel carbonyl :

V. P. in mm.	Mgrs. Ni per 100 grs. H <sub>2</sub> O		Calculated for 214 mm.*
214*	—	$\left\{ \begin{array}{l} 6.43 \\ 6.52 \\ 6.35 \end{array} \right\}$	6.43
186.5	5.53		6.34
138	3.92		6.09
183	5.64		6.59
109	3.32		6.32

\* Vapour tension of nickel carbonyl at 9.8° C.

The amount dissolved is directly proportional to the pressure, and these values plotted against the pressures as in Fig. 2 give a straight line.

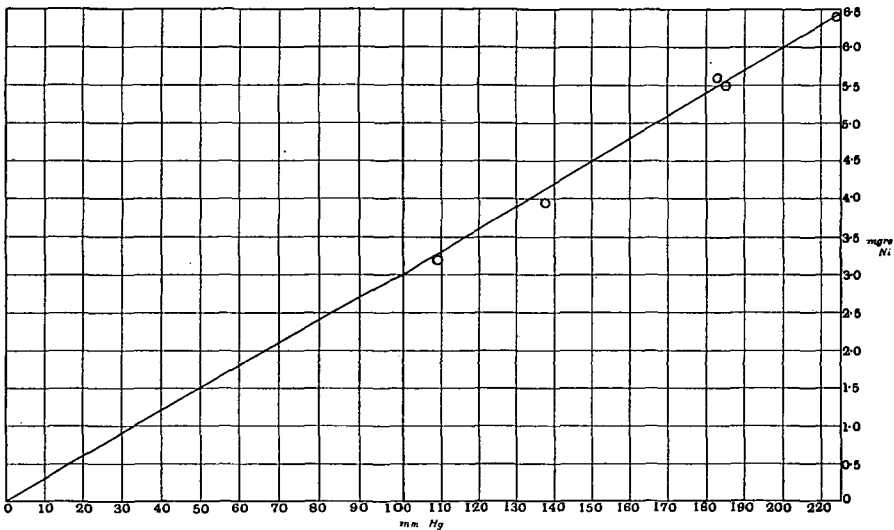


Fig. 2.

*Solubility of Nickel Carbonyl at Different Temperatures.*

For the determination of the solubility at varying temperatures, the method of saturating water with liquid nickel carbonyl and subsequently withdrawing a sample for analysis had to be employed for all temperatures above 10° C., owing to the quite appreciable dissociation of the gas which takes place leading to too high readings

of the partial pressure. The great disadvantage of employing excess of liquid carbonyl is the extreme precaution which has to be taken to avoid any minute particles of nickel carbonyl being carried over into the sampling flask.

The following results were obtained by the two methods employed :

1. Water shaken up with nickel carbonyl in excess.

Temperature	Mgrs. Ni per 100 grs.	Mgrs. Ni(CO) <sub>4</sub>	Ni(CO) <sub>4</sub> in c.c.ms.
9.8	6.42	6.43	18.01
	6.52		
	6.35		
22.3	4.77	4.69	13.63
	4.81		
	4.5		
29.1	4.41	3.82	11.1
	3.61		
	3.45		

2. Water brought into equilibrium with nickel carbonyl at various partial pressures.

Temp.	V. T. of Ni(CO) <sub>4</sub> at temp.	Part. P. of gas used	Mgrs. Ni per 100 grs. H <sub>2</sub> O	Calculated for V. T. at temp.
2.6	169	127	6.9	9.18
9.8	214	109	3.32	6.32
		138	3.92	6.09
		183	5.64	6.59
		186.5	5.53	6.34
23.4	363	250	2.13*	3.48
25	386	215.5	1.45*	2.6
35	564	132	0.38*	1.69
		199	0.48*	1.42
		235	0.7*	1.4

\* These results are certainly too low owing to dissociation.

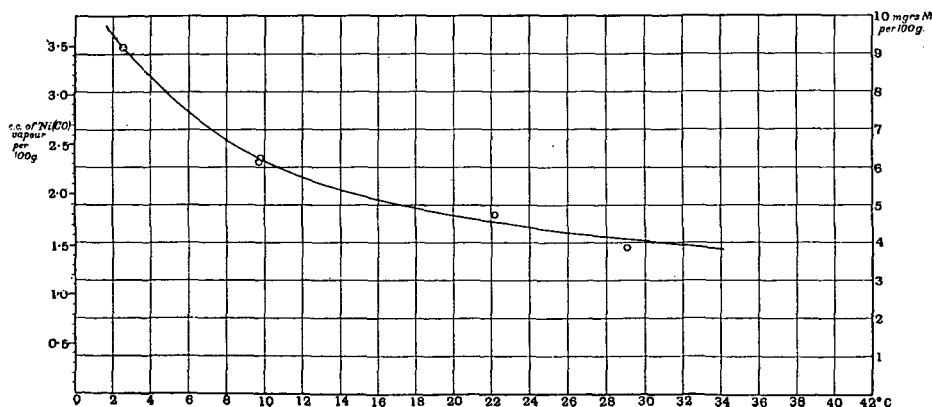


Fig. 3.

*Solubility of Nickel Carbonyl in Serum.*

The same methods as described above for water were attempted for serum, but it was found that in a viscid fluid the method with excess of liquid nickel carbonyl could not be relied on, since the inclusion of droplets of nickel carbonyl cannot be detected. The second method, therefore had to be resorted to. This, however, yields results which are too low at higher temperatures, on account of dissociation.

In displacing carbonyl from serum, a special gas washing bottle with the inside tube of capillary bore, only reaching to within a few millimetres above the level of the liquid, was used to prevent frothing. The displacement of the gas therefore took much longer than when hydrogen was bubbled through the liquid, and during this period small amounts of dissociation no doubt took place, as the temperature of the laboratory was between 15° and 20° C.

The results obtained are given in the following table and are set out graphically in Fig. 4.

*Solubility of Nickel Carbonyl at Different Temperatures.*

Temp.	V. T. of Ni(CO) <sub>4</sub> at temp.	Part. P. of gas used	Mgms. Ni per 100 grs. serum	Calculated for V. T. at temp.
1.1	157	112	16.1	22.57
10.0	219.5	139	10.07	15.71
23.5	364	186	4.66	9.0
35.0	568	209.5	2.71	6.81

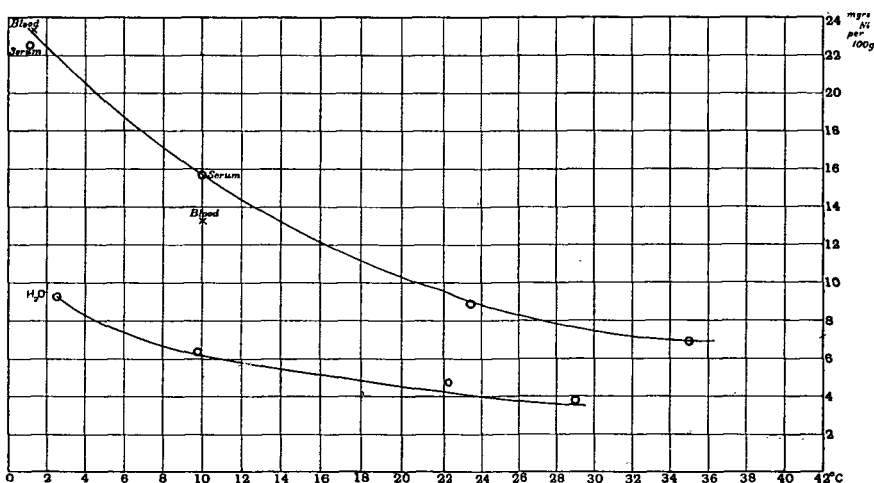


Fig. 4.

The solubility of nickel carbonyl in serum is higher than in water, and at body temperature the solubility in the former is about double that in the latter. In Fig. 4, the solubility curve in water is also plotted upon the same scale for comparison.

*Solubility of Nickel Carbonyl in Blood.*

At first it seemed not improbable that nickel carbonyl combined with the haemoglobin of the blood in virtue of its carbon monoxide groups, in which case the amount taken up by the blood would be greatly in excess of that dissolved in serum.

Langlois (1891) found that when blood was shaken up with nickel carbonyl the oxygen attached to the haemoglobin diminished by from 50% to 90%. He also injected rabbits with from 0.25 to 0.3 gr. of nickel carbonyl subcutaneously. The animals died within from 30 to 35 minutes. No oxygen was found attached to the haemoglobin after death. Lastly, he injected 0.3 gr. of nickel carbonyl into dogs, and obtained a great reduction of the oxyhaemoglobin. He therefore came to the conclusion that the oxygen was replaced by nickel carbonyl.

In an unpublished experiment, Shields was able to recover nickel carbonyl from blood by means of the gas pump. He concluded from this experiment that nickel carbonyl formed a chemical combination with a constituent of the blood.

In examining the behaviour of nickel carbonyl in blood it was thought advisable to proceed by carrying out parallel experiments with serum and blood. For this purpose, a litre bolt-head flask was fitted with a rubber cork, having four holes, to connect (1) with the filter pump, (2) with the mercury gauge, (3) with the nickel carbonyl burette and (4) with a T-shaped tube, leading to two equal 300 c.cm. flasks, one of which contained 100 c.cm. of blood and the other 100 c.cm. of serum. Each of the latter was provided with a capillary glass tube for removing samples of fluid. The flasks were exhausted, the serum and blood boiled vigorously *in vacuo* and the whole apparatus washed out four times with hydrogen, and again exhausted. Nickel carbonyl was run into the bolt-head flask until the mercury registered a pressure nearly equal to the vapour tension of nickel carbonyl at the temperature employed.

After a sufficient time had elapsed to ensure complete solution, samples of each were withdrawn into exhausted bottles and dealt with as in the previous experiments. While one sample was being analysed,

the other was kept at 3° C. in the cold room. As the displacing stage occupied some 2 to 2½ days, some dissociation possibly took place, even at the low temperature at which the flasks were kept. After the fluids were freed from nickel carbonyl, they were evaporated to dryness, ashed and the nickel content determined.

The results of two experiments are as follows:

1. Conducted at a temperature of 1·1° C.

Pressure of hydrogen before admitting Ni(CO)<sub>4</sub> ... 15·5 mm. Hg.  
Pressures when equilibrium was attained ... .. 127·5 mm.

Samples of the liquids were drawn off after 45 minutes. The nickel carbonyl in solution was determined as before.

The blood was dealt with first and yielded 17·35 mgrs. of nickel per 100 grs. which would correspond to 23·42 mgrs. of nickel per 100 grs. at the full vapour pressure of nickel carbonyl for the temperature employed.

The serum yielded 16·1 mgrs. nickel per 100 grs. or 22·57 mgrs. of nickel per 100 grs. calculated for the vapour pressure of nickel carbonyl at 1·1° C.

The blood further contained 2·64 mgrs. nickel in some form not displaceable with hydrogen and the serum contained 8·71 mgrs.

The following table expresses the result obtained, as mgrs. nickel per 100 grs.:

	Ni as Ni(CO) <sub>4</sub> mgrs.	Ni in solution mgrs.	Total mgrs. Ni
Serum	16·1	8·71	24·81
Blood	17·35	2·64	19·99

2. Conducted at a temperature of 10° C.

Pressure of hydrogen before admitting Ni(CO)<sub>4</sub> ... 25 mm. Hg.  
Pressure when equilibrium was attained ... .. 164 mm.

Samples were taken after 45 minutes. The serum was first dealt with, and the results were as follows:

	Ni as Ni(CO) <sub>4</sub> mgrs.	Ni in solution mgrs.	Total mgrs. Ni
Serum	10·07	8·27	18·24
Blood	8·53	11·32	19·85

Since the fluid dealt with first in each case gave higher results, it is probable that the amount of nickel carbonyl found in the second fluid was too low on account of dissociation having taken place. One is therefore justified in concluding that at 1·1° C. and 10° C., the amount



of nickel carbonyl taken up by blood is about the same as that by serum, and that the values, calculated for the full vapour pressure of nickel carbonyl at these temperatures, are about 23 and 15 mgrs. nickel per 100 grs. respectively. It is not obvious why the two results should be so nearly the same, for the physical and chemical properties of blood and serum differ very considerably.

It is therefore unnecessary to come to the conclusion, as Langlois and Shields have done, that a chemical combination between haemoglobin and nickel carbonyl is formed, as the total quantity of nickel carbonyl which blood is capable of taking up can exist in solution in serum. The oxygen of the haemoglobin is of course replaced by any carbon monoxide which may be liberated by dissociation of the nickel carbonyl. Langlois used quantities of nickel carbonyl which would yield sufficient carbon monoxide after dissociation to saturate the haemoglobin.

*On the Properties of the Substance formed by the Dissociation of Nickel Carbonyl in Water in the Presence of Air.*

When nickel carbonyl is dissolved in water and dissociation takes place, the condition of the nickel depends on the presence or absence of oxygen and carbon dioxide. In the entire absence of both of these, a precipitate of metallic nickel is formed, which can easily be removed by sedimentation or by filtration. When air is present, the nickel appears as a fine greenish white precipitate in a turbid fluid.

The precipitate is probably a hydrated sub-carbonate (Mond), but the exact composition has not been determined. The turbidity of solutions in which decomposition has occurred cannot be removed by passing the liquid through a paper filter, but when it is passed through a Berkefeld filter a clear filtrate is obtained, which, however, contains nickel in solution. The maximum quantity of nickel which was found in water after dissociation of nickel carbonyl had taken place and the carbonyl and undissolved salts had been got rid of by filtration through a Berkefeld filter was 3.47 mgrs. nickel per 100 c.cm. of water.

The properties of this nickel solution have been compared with those of nickel hydrate and carbonate. The differences are best understood by placing the behaviour of each side by side, and, for the sake of brevity, the product of dissociated nickel carbonyl will be referred to as *dissociation product*.

	Solubility	Filtration	Effect of heat	Effect of addition of Electrolytes (NaCl)
Ni(OH) <sub>2</sub>	Practically insoluble (less than 1/100 mgr. in 10 c.cm. of water).	When water is shaken up with Ni(OH) <sub>2</sub> and passed through any filter paper, the filtrate is quite clear.	Unaltered as regards solubility after boiling.	No solution exists.
NiCO <sub>3</sub>	Is soluble to the extent of 0.48 mgr. Nickel per 100 c.cm. of water at room temperature (18 to 23°C.).	When water is shaken up with Nickel Carbonate, the filtrate is only clear if a close filter is used.	Boiling deposits a trace of precipitate containing Nickel. The filtrate contains the greater bulk.	Electrolytes do not precipitate the salt from solution.
<i>Dissociation Product</i>	Is dissolved to the extent represented by 3.47 mgrs. Nickel per 100 c.cm. water at room temperature.	When water is shaken up with Nickel Carbonyl and the Carbonyl is removed, the liquid can only be cleared of its turbidity by passing through a Berkefeld filter.	From the solution of <i>Dissociation Product</i> , the Nickel is almost completely precipitated by boiling. The precipitate can be completely removed by filtering through paper, leaving the merest trace of Nickel in solution.	The addition of Electrolytes to solution of <i>Dissociation Product</i> precipitates some of the Nickel. When a saturated solution of NaCl is added, about 25% of the Ni is precipitated, while when (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> is added, rather less is thrown out of solution.

Nickel hydrate is slightly soluble in its mother liquor, *i.e.* sodium hydrate; nickel carbonate is more soluble in its mother liquor, *i.e.* sodium carbonate, and the fluid becomes slightly turbid, which turbidity can only be removed by filtration through a close filter paper.

When carbonic acid is passed through a solution of nickel carbonate, and the liquid filtered through a close filter paper, the solubility is found to be slightly diminished (0.41 mgr. against 0.48 mgr. nickel per 100 c.cm. of water).

After standing for a few days, the solution of *dissociation product* becomes opalescent, and after the 5th day or later a fine precipitate separates out. This precipitate contains nickel.

The solution of *dissociation product* was subjected to dialysis and it was found to pass through the membrane in 36 hours. 74 c.cm. of a solution containing 1.1 mgr. nickel per 100 c.cm. were placed inside the skin and 1500 c.cm. of water were placed outside. The dialysate contained 0.96 mgr. of nickel. A solution of *dissociation product* containing 0.15 mgr. in 50 c.cm. was dialysed for 12 hours with 800 c.cm. of water. At the same time, a solution of nickel carbonate of the same strength

was also subjected to the same procedure. The latter was found to have dialysed completely, while the dialysis in the former was not complete. The dialysate contained 0.03 mgr. of nickel, but the fluid inside the membrane only contained 0.01 mgr., so that the remainder was absorbed by the membrane. A solution of *dissociation product* containing 0.41 mgr. per 100 c.cm. was next dialysed for seven hours. It was found that roughly one-third of the same strength solution of nickel carbonate passed through the membrane in this time, the rest being found in the fluid inside the skin. In the case of the *dissociation product* although about 0.02 mgr. of nickel was still in the fluid in the skin, only a mere trace could be found in the dialysate. The greater part of the nickel had therefore been absorbed into the membrane.

*Solubility of Dissociation Product in Serum and its Relation to the Proteid Content.*

Serum was saturated at 35° C. with nickel carbonyl (*a*) in the absence of air and (*b*) in the presence of air, and was then subjected to various investigations. The serum in the former case was boiled *in vacuo*, but this is insufficient to remove all the carbon dioxide. The nickel carbonyl having been removed, the serum was filtered through a Berkefeld filter. The resulting filtrate was quite clear, but the colour varied in consequence of concentration behind the filter.

1. Experiments conducted with serum which had been boiled *in vacuo* and the oxygen removed.

Various samples were analysed for nickel and also for nitrogen (Kjeldahl).

The results were as follows :

	Mgrs. Ni per 100 grs. serum	N per 5 c.cm., expressed as $\frac{2}{10}$ NH <sub>3</sub>
1.	2.98	13.7
2.	3.1	18.7
3.	2.18	14.7
4.	1.3	4.7
5.	2.48 (?)	5.2

The figures show no proportion between nickel and nitrogen, so that a chemical compound is excluded.

2. Experiments conducted in the presence of air.

Serum, serum diluted with water, and water were saturated with nickel carbonyl, kept at 35° C. for one hour and then placed in the cold room for 20 hours. The nickel carbonyl was driven off by bubbling air

through the liquids, and the liquids were passed through a Berkefeld filter. Samples ashed gave the following values.

1. Pure Serum	contained	13.33 mgrs. Ni per 100 grs.
2. Serum + water (1 : 1)	„	7.75 „ „ „
3. „ „ (1 : 2)	„	7.25 „ „ „
4. „ „ (1 : 4)	„	6.19 „ „ „
5. Pure water	„	0.72 „ „ „

Here again no ratio between nickel and nitrogen (dilution of serum) is to be found.

#### *Dialysis.*

Serum treated as before in the absence of oxygen was subjected to dialysis for 24 hours.

- 15 grams of Serum inside the skin and 1400 c.cm. H<sub>2</sub>O outside.  
Dialysate contained 0.3 mgrs. Nickel.  
Fluid inside the skin „ 1.4 „
- 25 c.cm. of Serum and 1315 c.cm. Water.  
Dialysate contained 1.57 mgrs. Nickel.  
Fluid inside the skin „ 0.63 „
- 40 c.cm. of Serum and 1500 c.cm. of Water.  
Dialysate contained 2.65 mgrs. Nickel.  
Fluid inside the skin „ 1.22 „

The serum in 2 and 3 was not quite fresh and allowed some coagulable material to pass through the membrane, but the results show that the nickel is only partially dialysable.

#### *Discussion of the Condition in which Nickel exists in Serum after Dissociation of Nickel Carbonyl has taken place.*

The nickel derived from the dissociation of nickel carbonyl in serum in the presence of oxygen and carbon dioxide exists partly in solution and partly as a finely divided precipitate. The latter can only be removed by filtering through a Berkefeld filter. The dissolved nickel does not stand in any proportion to the nitrogen of the serum. The maximum quantity found dissolved in serum expressed as nickel was 13.33 mgrs. per 100 grs. of serum. Solutions of this *dissociation product* are only partly dialysable.

The increased solubility in serum as against water must depend either on the presence of inorganic and organic salts, or on the presence of proteid and other colloidal matter (*e.g.* pigment) or both.

In order to decide to what conditions this increased solubility is

due, experiments with nickel hydrate, nickel carbonate and *dissociation product* in various solvents were carried out.

Serum was filtered through a Martin Gelatin filter, and a filtrate was obtained which practically represented the salts of serum dissolved in the water of serum. 100 c.cm. of this solution dissolved 5.04 mgrs. of nickel as *dissociation product*. The same quantity of the solution dissolved 4.15 mgrs. of nickel in the form of nickel carbonate.

The solutions in the salts of serum solution however became turbid after two days. The turbidity could not be removed by filtration through the closest filter paper.

A fresh solution of sodium phosphate ( $\text{Na}_2\text{HPO}_4 + 12\text{H}_2\text{O}$ ) containing 0.25 gr. to the litre was next used, as representing the minimum value of phosphates in serum (Hammersten gives this at 0.05 gram pentoxide of phosphorus ( $\text{P}_2\text{O}_5$ ) per litre). The solution was saturated with carbon dioxide to correspond more nearly to the condition of serum. It dissolved 2.45 mgrs. of nickel as *dissociation product* and 12.3 mgrs. of nickel as nickel hydrate per 100 c.cm.

Nickel carbonate was also shaken up in the phosphate solution and was found to be practically insoluble.

The solubility of *dissociation product* in solutions of sodium carbonate is approximately the same as that in water.

Since serum dissolves *dissociation product* to the extent of 13.3 mgrs. of nickel per 100 grs. of serum, and whereas water solutions of phosphate of sodium and solutions of the salts of serum dissolve considerably less, there is a balance of dissolved nickel yet to be accounted for.

Serum dissolves freshly precipitated nickel hydrate.

Nickel hydrate freshly precipitated was added to serum, well shaken up at room temperature and filtered through a close filter paper. The filtrate which was quite clear contained 22.5 mgrs. of nickel per 100 c.cm. The serum was not freed from oxygen or carbon dioxide. The unashed filtrate gave the dimethylglyoxime reaction. Since serum to which nickel sulphate and an excess of potassium hydrate are added (*i.e.* potassium nickel albuminate) does not give this reaction, one can deduce that the nickel therefore existed in the filtrate as free ions.

The solubilities of nickel hydrate, nickel carbonate and *dissociation product* are given in the following table for comparison. The solubility of *dissociation product* is inconstant, and depends upon the temperature at which the nickel carbonyl is dissociated. The lower the temperature

at which dissociation takes place, the greater is the amount of material which goes into solution in the water.

The figures given are therefore only to be regarded as maximum values obtained.

	H <sub>2</sub> O	Na <sub>2</sub> HPO <sub>4</sub>	Salts of serum sol.	Serum
Ni(OH) <sub>2</sub>	0	12·3	—	22·5
NiCO <sub>3</sub>	0·48	0	4·5	16·03
<i>Dissociation Product</i>	3·47	2·45	5·04	13·3

It was thought that if the proteid material combined with nickel, this compound might be thrown down by precipitating the proteids with ammonium sulphate.

25 c.cm. of serum in which *dissociation product* was dissolved was used. Ammonium sulphate was added to this to produce a 33% saturation. The precipitate was collected and ashed and the ashed material is called (*a*) below. Next, sufficient ammonium sulphate was added to the filtrate to produce a 50% saturation. The ashed precipitate is called (*b*). The filtrate was saturated with solid ammonium sulphate. The precipitate ashed is called (*c*). The filtrate of this was boiled and the precipitate and filtrate ashed are called (*d*) and (*e*) respectively.

( <i>a</i> )	Containing	0·12 mgrs. Ni or 0·49 mgrs. Ni per 100 grs.
( <i>b</i> )	„	about 0·01 „ or 0·04 „ „ „
( <i>c</i> )	„	0 or 0 „ „ „
( <i>d</i> )	„	0 or 0 „ „ „
( <i>e</i> )	„	1·27 „ or 5·1 „ „ „

This experiment was further compared with the following :

Serum was shaken up with nickel carbonate and filtered through a close filter paper. The filtrate was quite clear. Ammonium sulphate was then added as before.

The results were as follows :

( <i>a</i> )	Contained	6·7 mgrs. Nickel per 100 grams Serum.
( <i>b</i> )	„	4·7 „ „ „ „
( <i>c</i> )	„	0·9 „ „ „ „
( <i>d</i> )	„	0 „ „ „ „
( <i>e</i> )	„	4·9 „ „ „ „

In the case of *dissociation product* the small fraction thrown down may have been due to the action of electrolytes, but the reason why nine-tenths should remain in solution is not clear. In the second case, while the action of electrolytes cannot be excluded, the results seem rather to suggest that a part of the nickel had entered in combination with the proteid.

The facts that when in aqueous solution, nickel is precipitated by boiling, and by adding electrolytes, and that at a certain stage of dialysis, adsorption into the membrane takes place, and also that the difficulty of obtaining a constant strength of the aqueous solutions may be due to the substance not being in ordinary solution, are strongly in favour of the view that *dissociation product* passes into colloidal solution.

The increased solubility of *dissociation product* in serum can also be explained on this view. The influence of serum in keeping metallic precipitates in colloidal solution is well known. When copper sulphide, for example, is formed in serum, it does not precipitate in the usual way but forms a colloidal solution.

The increased solubility of *dissociation product* in serum can be explained in this way. At present, however, it is impossible to exclude the possibility of a proteid combination, but this would at most only affect a small proportion of the dissolved nickel. That the major part of the nickel is not combined with the proteid has already been shown.

To sum up, nickel carbonyl dissociates in the presence of oxygen and carbon dioxide into carbon monoxide and a nickel compound, which is probably a hydrated basic carbonate. This substance is slightly soluble in water (0.0035 %) and considerably more soluble in serum (0.0133 %). When this substance is formed in water or in serum it exists partly in a state of extremely fine division, and this causes a turbidity of the liquid to appear. The particles are so small that filtration through a Berkefeld filter is necessary to clear the liquid.

A comparison with nickel hydrate and nickel carbonate shows that the substance obtained from the dissociation of nickel carbonyl is not identical with either of these, as they exist when precipitated in the ordinary way. The solutions in water and in serum appear to be colloidal solutions. From the aqueous solutions the substance can be precipitated by heat, and to a certain extent by the action of electrolytes, and a precipitate forms spontaneously after standing for some days.

The solubility of this substance in solutions of the salts of serum and in solutions of sodium phosphate does not account for its relatively high solubility in serum. There is no evidence of the existence of a proteid combination.

## SUMMARY AND CONCLUSIONS.

1. Nickel carbonyl is a highly toxic compound.
2. Dissociation of nickel carbonyl takes place rapidly at the temperature of the body in the presence of air, moisture and carbon dioxide.

Nickel carbonyl also dissociates at the temperature of the body in the absence of air, moisture and carbon dioxide, but not to the same extent as in the presence of these owing to the fact that a reversible action occurs between the nickel and the dissociated carbon monoxide.

3. Nickel carbonyl is soluble in water to the amount of 2.36 c.cm. of vapour (*i.e.* 6.43 mgrs. of nickel) per 100 grs. at 9.8° C.

The amount dissolved in water is directly proportional to the pressure, when the temperature is constant.

The solubility in water diminishes as the temperature is raised.

4. The solubility of nickel carbonyl in serum and in blood is about 2½ times greater than that in water. At 10° C. 100 grs. of serum dissolves 5.98 c.cm. of nickel carbonyl vapour (*i.e.* 15.71 mgrs. of nickel).

5. When nickel carbonyl is brought into contact with water, serum, blood and other liquids, if oxygen and carbon dioxide are present, dissociation occurs and a substance is formed, probably a hydrated basic carbonate of nickel, which is slightly soluble, but also forms a very fine precipitate, which renders the liquid turbid. The liquid can only be cleared of the turbidity by passing it through a Berkefeld filter.

6. Water dissolves about 0.0035 % of the product of dissociated nickel carbonyl (reckoned as nickel) at 18° C. About the same amount is dissolved in 10 % solutions of sodium carbonate.

The solubility of this product of dissociated nickel carbonyl is greater in serum than in water. Serum dissolves about 0.0183 % (reckoned as nickel). A solution of the salts of serum, under the same conditions, dissolves 0.005 % (reckoned as nickel). Solutions of sodium phosphate of approximately the same strength as the phosphates in serum, dissolve 0.0025 % (reckoned as nickel).

7. Nickel hydrate is insoluble in water but is soluble in serum to the extent of 0.0225 gr. nickel per 100 grs. serum, and in sodium phosphate solutions to the extent of 0.012 % (reckoned as nickel).

8. Nickel carbonate is soluble in water to the extent of 0.0005 % (reckoned as nickel), and in serum to an amount, corresponding to 0.016 gr. % of nickel. Salts of serum dissolve 0.0045 gr. % of



nickel carbonate reckoned as nickel. Nickel carbonate is insoluble in sodium phosphate solutions.

9. *Dissociation product* in aqueous solution is precipitated by boiling, and to a certain extent by the addition of electrolytes. Some of it is thrown out of solution spontaneously on standing for some days, whereas solutions of nickel carbonate are not precipitated by either of the above means.

10. Solutions of *dissociation product* dialyse more slowly than solutions of similar strength of nickel carbonate, and at a certain stage in the dialysis the greater part of the nickel is adsorbed into the membrane.

11. No constant ratio exists between the nickel and the nitrogen contents of solutions of *dissociation product* in serum of different strengths.

12. When the proteids of the serum, containing the product of dissociated nickel carbonyl in solution, are precipitated by ammonium sulphate, nine-tenths of the nickel remains in solution. One-tenth is precipitated by adding sufficient ammonium sulphate to produce one-third of saturation, and about an additional one-hundredth by producing one-half of saturation.

13. The product of dissociated nickel carbonyl when dissolved in serum is only incompletely removable from solution by dialysis.

The product of dissociated nickel carbonyl is therefore not identical with nickel carbonate or nickel hydrate, and appears to exist in a condition of colloidal solution.

14. The poisonous properties of nickel carbonyl do not depend, as was at first supposed, on the carbon monoxide of the compound.

15. Nickel carbonyl when mixed with air and inhaled by an animal, cannot be absorbed as such, as it becomes split up into the nickel containing substance (?hydrated basic carbonate of nickel) and carbon monoxide, before or soon after reaching the alveoli of the lungs.

16. The poisonous effects of nickel carbonyl are entirely due to the nickel of the compound. The peculiar toxicity of the compound is due to the fact that being introduced in a gaseous form and that the nickel is deposited as a slightly soluble compound in a very fine state of subdivision over the immense area of the respiratory surface.

In Part II. the passage of the nickel through the body from its deposition on the respiratory surface to its ultimate excretion will be followed out.

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