THE TOXICOLOGY OF NICKEL CARBONYL. PART II.

Plates VII—IX, and Four Figures.

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I. INTRODUCTION.

Nickel responsible for the symptoms of Nickel Carbonyl poisoning.

IN Part I¹ it was shown that the toxic symptoms after nickel carbonyl inhalation are not due to either the nickel carbonyl as such or to the carbon monoxide which is given off when the compound is exposed to air and moisture at body temperature. It was therefore concluded that they are occasioned by the absorption of the nickel set free and that nickel carbonyl poisoning is a particular case of nickel poisoning.

The fact that the nickel is deposited over the immense surface of the lungs in a condition which is especially favourable for absorption renders a study of the toxicology of this compound of great interest.

The results of this second part of the investigation will be presented in the following order :

1. Further evidence that the nickel is responsible for the symptoms, by comparison of the course of poisoning and of the changes induced by the inhalation of nickel carbonyl vapour with the course of poisoning and changes induced by the administration of other nickel compounds.

2. An attempt to trace the nickel through the organism.

3. A comparison of the toxic effects of nickel, iron and cobalt.

¹ See *Journ. of Hygiene*, VII. 4, pp. 525—551. 1907.

II. COURSE OF POISONING IN ANIMALS.

(i) *Methods employed.*

The experiments with nickel and iron carbonyl were, save for a few of the earlier ones, in which another method which did not prove satisfactory was employed, carried out as follows:—the liquid carbonyl was placed in a graduated burette of 1 c.c. capacity (see Plate IX *e*) provided with an ordinary stopcock above and a three-way stopcock below. The lower end of the burette was passed through a rubber cork, fitted into a glass receiver (*f*) which contained a little cotton wool to catch the carbonyl. A given quantity of the carbonyl was run out of the burette by turning on the three-way stopcock. The tap having been reversed, a small quantity of carbon dioxide was allowed to pass through the receiver containing the nickel carbonyl into a large gasholder (A), the capacity of which was approximately 240 litres so as to evaporate the carbonyl in the receiver. A little air was blown into the gasholder before the carbon dioxide was turned on, to prevent the carbonyl condensing in the latter. While the carbon dioxide was blowing all the carbonyl over, air was introduced into the gasholder through the second tap (*d*). The quantity of carbonyl being known (1 c.c. of liquid nickel carbonyl yields 181.1 c.cm. of vapour at 0° C. and 760 mm. Hg., and 1 c.cm. of iron carbonyl yields 167.39 c.cm. of vapour) and the capacity of the gasholder having been determined and recorded on a scale (*c*) attached to the upright, the volume percentage of the carbonyl in the air could be accurately measured. After all the fluid had been evaporated, the taps of the gasholder were closed and the heavy weights exchanged for one of two kilograms. The carbonyl burette was removed and the chamber (B) was connected to the gasholder by applying the tube *g* to the tap *b*. The chamber was an air-tight box, made of mahogany and glass with rubber stoppings adjusted to the windows and lid. From the outlet (*h*) a tube carried off the vapour and air to a Bunsen burner, where all the carbonyl was dissociated. A screw clamp applied to this tube regulated the flow of the vapour mixture passing through the chamber. The rate was determined by timing the descent of the level of the top of the gasholder against the scale (*c*). It was found that a satisfactory rate for the purpose was three litres per minute.

The poison chamber contained air at the beginning of each experiment, and this air was gradually replaced by the vapour mixture in the

gasholder. In order to determine the rate of replacement, test experiments were conducted with oxygen (in which Dr Boycott, of Guy's Hospital, kindly assisted me) and the gas issuing from the outlet was analysed at short intervals. The first series of determinations were conducted with an inanimate object in the chamber in the place of the rabbit. The second series was conducted with a living rabbit, and it was found that the mechanical action of the respiration, etc. on the mixing of the gases was appreciable. With the rate of flow employed the quantity of oxygen absorbed by the rabbit may be neglected. It was therefore considered better to utilise the results of the second series alone, and from the figures obtained a curve of the rate of displacement was interpolated, the ordinates representing the percentage of vapour mixture which has replaced the air in the chamber and the abscissa representing the time in minutes. The figure lying above the curve has been plotted as a rectangular figure, and from this it appears

Fig. 1. Curve showing Rate of Displacement in chamber.

that a deduction of $14\frac{1}{2}$ minutes would be a necessary correction in taking the time during which the animal was in the poison chamber as indicating the opportunities for intake of nickel carbonyl vapour. The curve and rectangular figure is given in Fig. 1).

It must however be pointed out that nickel carbonyl would not behave like a stable gas, such as carbon monoxide, on account of its

ready dissociability in the presence of air and moisture at the temperature of the animal body (see Part I) and the results obtained by means of this curve are only approximate.

(ii) *Concentration of vapour and length of exposure.*

A large number of the experiments with nickel carbonyl are available for the purpose of determining how much of the vapour has to be inhaled to produce death. Naturally some individual idiosyncrasy played a considerable rôle in the inhalation experiments. One hundred and twenty-two rabbits were exposed to atmospheres containing varying volume percentages of nickel carbonyl vapour in air for varying periods. After a few preliminary experiments, the time factor was kept constant in a long series and the concentration was varied. When inhaled for 65 minutes, calculated from the time of establishing a connection between the gasholder and the poison chamber, 0·018 to 0·0188 volume per cent. of nickel carbonyl in air killed 64 out of 77 rabbits, i.e. 83·11 %. It would thus appear that, after making the 14½ minutes allowance as suggested above, 0·018 volume per cent. was capable of killing when inhaled for 50½ minutes. Variations of the time and concentration factors led to the following results :

TABLE I.

	Concentration of Ni(CO)	Ratio of concentration to 1st series	Length of whole experiment	Time after deduction of 14½ minutes	Result
1.	0·018 vol. %	100 : 100	65	50·5	†
2.	0·04 „	222 : 100	40	25·5	R
3.	0·075 „	416 : 100	31	16·5	R
4.	0·016 „	88 : 100	72	57·5	†
*5.	0·015 „	83 : 100	108	93·5	†
6.	0·0136 „	72 : 100	79	64·5	R
7.	0·0134 „	74 : 100	82	67·5	†

* This was one single rabbit and the time was unnecessarily long.

† signifies died. R signifies recovered.

From these figures it will be seen that the concentration of vapour necessary to kill an animal was in inverse proportion to the time the vapour was inhaled.

Cats withstand the effect of the vapour better than rabbits. After inhaling the carbonyl for 90 minutes (i.e. 75·5 minutes after making the deduction) 25 out of 30 animals, i.e. 83·3 %, died when 0·04 volume per cent. of the vapour in the air was used.

Dogs die after inhaling air containing 0.036 volume per cent. of nickel carbonyl for 90 minutes (i.e. 75.5 minutes after the deduction is made).

(iii) *Course of poisoning.*

The effect of the inhalation of the vapour of nickel carbonyl by rabbits may be illustrated by the following account of a typical case.

Rabbit 12 (second series). Weight 1935 grams. Temp. 39.4° C.

0.2 c.cm. of liquid nickel carbonyl was evaporated into the gasholder and to the total quantity of air made up to 192.75 litres, i.e. 0.0188 volume per cent.

The vapour mixture was allowed to pass through the chamber at 10.20 a.m. The respiratory rate was increased after 10 minutes to 132 in the minute, probably on account of apprehension, but soon fell again to 90. After 25 minutes, some uneasy movements were performed. After 34 minutes, the respiration was 176 and the rabbit became extremely restless. The respiration increased in rate steadily from this time and dyspnoea was seen. The restlessness passed off. At the end of one hour, the respiratory rate was 180, the rabbit passed urine and faeces but remained quiet. The visible vessels in the ears did not show any change of colour, but the lips and tongue were cyanotic.

The rabbit was taken out at 11.25, i.e. after having been in the chamber for 65 minutes.

At 11.30, the respiratory rate was 180, the dyspnoea had disappeared and the animal appeared comfortable. The temperature taken at 2.45 p.m. was 38.8° C. and the respiratory rate 120. During the rest of the day the rabbit seemed to be but little affected.

On the following morning the weight had decreased to 1712 grams, the temperature was 38.0° C., the respiratory rate was 196; there was dyspnoea, slight cyanosis of the lips and tongue, and on ausculting the chest, a prolongation, roughening and loudening of the inspiration was heard over the inter-scapular space and also to a less degree in the axillae. This gave the impression of reversed bronchial breathing. The expiratory sounds were unaltered and no râles were heard. The rabbit was taking very little food, but on being allowed out of its cage, was quite lively. During the afternoon, the dyspnoea increased and on the following morning (i.e. 48 hours after the inhalation) it seemed very ill. The weight was 1673 grams, the temperature 38.3° C., and the respiratory rate 204. There was much dyspnoea and cyanosis. It refused food altogether. The physical signs had increased considerably. 24 hours later a distinct improvement was seen, the respiratory rate was 156 and the dyspnoea was not so marked, while the temperature was 39.4° C. The weight however was 1607 grams. At the end of 96 hours, the weight had dropped to 1570 grams, i.e. a loss of nearly 19% of its original weight. The respiratory rate was 132, there was urgent dyspnoea again and extreme cyanosis. The temperature had fallen to 34.5° C., and the animal was apathetic and ill. The physical signs during the last 24 hours revealed consolidation of both lungs, but there was no absolute dulness to percussion. The character of the breath sounds

was more tubular than at first, and there were a few râles to be heard. It died at 1 p.m. i.e. 98½ hours after poisoning.

The following chart shows the variations of temperature and respiratory rate.

Fig. 2. Chart of Rabbit 12.

(iv) *Variations.*

In other cases, some variations of the symptoms were noted. In cases in which the death took place late, the respiratory rate became very slow but the dyspnoea increased up to the end. In one instance a rabbit died after 204 hours. During the last 12 hours of life, the respiratory rate was only 24 in the minute. When the inhalation was prolonged for a considerable time, and when strong concentrations of vapour were used, the lung symptoms became more intense.

The heart was rarely affected, the rate however usually failing to keep pace with the respiratory rate. Reduplication of the first sound at the apex was heard at times. The peripheral vessels were frequently contracted from the third day onward.

As a rule, symptoms referable to disturbance of the central nervous system were not well marked. It is difficult to decide whether the dyspnoea and cyanosis were entirely due to the changes in the lungs. In a few cases, there was paresis of the hind legs. The muscular tone

was diminished or lost, the muscle most frequently affected being the sphincter ani. From the second day onwards there was loss of sexual desire. Urine was retained for some hours before death in a number of cases. Final convulsions were frequently seen. The animals nearly always assumed an orthotonic position before death.

The temperature curve was of the type shown above in the majority of the rabbits. Modifications however were seen, including the following:

- (a) gradual fall after 24 to 36 hours,
- (b) sudden fall at 24 hours to 35° C. or lower,
- (c) fall within 18 hours, with subsequent rise and later fall.

Death took place between 60 and 90 hours after poisoning in a large number of experiments. The average for the 64 rabbits poisoned by inhaling 0.018 volume per cent. of nickel carbonyl for 50½ minutes was 69.2 hours.

The variations noted included:

- (a) Immediately after poisoning (1 in 122).
- (b) Between 23 and 36 hours (11 in 122).
- (c) Between 100 and 150 hours (4 in 122).
- (d) Over 200 hours (2 in 122).

Some rabbits appeared to be very resistant toward the effect of the vapour and this resistance appears to vary at different times in the same animal. A previous non-fatal poisoning dose appears to render the rabbit slightly less susceptible to the vapour, provided that sufficient time is allowed to elapse for the obvious lesions to be repaired. Independent diseases, such as tuberculosis, render rabbits more susceptible to nickel carbonyl.

In Part I of this paper, reference was made to the examination of the blood for carbon monoxide. The maximum amount of carbon monoxide found in any one case was equivalent to a saturation of not more than 5 % of the haemoglobin.

A number of rabbits were poisoned with quantities of nickel carbonyl just sufficient to produce death, and the blood was examined daily at the same time. It was found necessary first to control the blood counts for several days, prior to the poisoning, since some rabbits exhibit considerable variations. Only those rabbits which gave steady counts were used. The number of red corpuscles remained unaltered until the 4th or 5th day, when a reduction to about $\frac{2}{3}$ of the original number was observed. The haemoglobin value fell at about the same time, but the lowest values were only about 85 % of the full values.

During the first three days, the leucocytes tended to decrease in number but never reached below the lower limit of a normal count. The slight drop was followed by a recovery to the former level. Just before death a distinct leucocytosis was seen. The polymorphonuclear elements were the only leucocytes increased in number. A curve is appended to illustrate the type of the changes (Fig. 3).

Fig. 3.

(v) *Poisoning in cats.*

The following is an example of the typical course of nickel carbonyl poisoning in cats :

Cat 11. Weight 3340 grams.

0.42 c.cm. of liquid nickel carbonyl was evaporated into the gas holder and the total quantity of air was made up to 189.8 litres, i.e. 0.04 volume per cent.

During the inhalation there was preliminary increase of the respiratory rate, followed by ordinary breathing, and after about 25 minutes, rapid and difficult breathing was evidenced. Toward the end of the inhalation, faeces were passed and the cat vomited. There was salivation. On being taken out at the end of 90 minutes, it was obviously very giddy, dyspnoeic and somewhat cyanotic. At 4 p.m. the temperature was 39.4° C., the respiration was 42 in the minute and the heart was beating 180 in the minute. It was still distinctly affected from the

inhalation but the dyspnoea was markedly less than it was when the cat was taken out of the poisoning chamber. On the following day the weight was 3170 grams, the temperature 38.5°C ., the respiration 66 and the heart beat 156. There was some dyspnoea and the cat was dull and apathetic. The inspiratory sounds were harsh and loud all over the chest, but there were no added sounds, and no impairment of resonance. The dyspnoea increased during the day, and on the following morning was very marked. The weight had then dropped to 3118 grams, the temperature was 36.8°C ., the respiratory rate 114 and the heart beat 102. The physical signs all over both lungs were typical. On the next day the weight still further decreased to 3010 grams, the temperature was 35.2°C ., the respiration 78 and the heart beat 132. The reflexes were distinctly increased, the pupils were dilated but reacted to light, and there was marked cyanosis. Over the right lung, behind, there was impairment of resonance and the expiratory murmur was rough and prolonged in this situation. Otherwise the signs were unaltered. During the following days the weight decreased about 100 grams a day, while the respiratory rate decreased to 54, 30, 24, 22 and 23 on the successive days. The heart rate remained about 144 during the latter part of the illness. The temperature rose on the 5th day to 37°C ., fell slightly on the 6th but rose again on the 7th day to 37.4°C . After this it fell to 33°C . and 31°C . before death. A curve showing the respiration, pulse rate and temperature is appended.

Fig. 4. Chart of Cat 11.

On the 6th day the reflexes became less brisk, and on the 7th day there was distinct paresis with some ataxy of the gait. The signs of consolidation did not increase and on the 7th day a considerable increase in the râles was noted. The

breath sounds became faint. The cat died after about 200 hours, during the night of the ninth day. The loss of weight represented over a quarter of the original weight. There was diarrhoea during the last three days.

(vi) *Variations of symptoms in cats.*

Vomiting during the inhalation was frequently seen. In some cases there was little increase in the respiratory rate during the greater part of this period.

The respiratory rate in the subsequent stages varied considerably. The following gives the range of the variations :

Up to 6 hours after poisoning		26 to 30	per minute.
7 to 24 hours	„ „	 30 to 48	„ (or more)
25 to 48	„ „	 60 to 120	„
49 to 60	„ „	 70 to 100	„
61 to 84	„ „	 70 to 120	„
85 to 96	„ „	 70 to 100	„
97 to 120	„ „	 60 to 80	„

The decrease in the rate was marked in some cases, especially when death occurred late. When recovery took place the fall to normal was gradual. During the acute period, laryngeal stridor and a hoarse miaow were often observed.

Death occurred on an average in 88·93 hours in cats which were subjected to the inhalation of 0·04 volume per cent. for 75·5 minutes.

In 1 case, it was delayed to 348 hours.

In 3 cases, it took place between 200 and 250 hours.

In 6 cases, it took place between 100 and 200 hours.

In 4 cases, it took place between 27 and 40 hours and in one case it took place during the inhalation.

(vii) *Poisoning in dogs, guinea-pigs, etc.*

Dogs behave like cats ; they are slightly more susceptible to the poison than the latter. Diarrhoea was present in all the cases. In other respects no differences were noted.

Guinea-pigs proved to be very susceptible to nickel carbonyl vapour. They showed most of the signs and symptoms which are seen in rabbits.

Insects die rapidly in dilute mixtures of the vapour.

A series of experiments was conducted on frog's heart and muscle nerve preparations. The carbonyl was applied in vapour and also in liquid form. No direct effect could be noted. It is however uncertain whether the nickel carbonyl ever reached the muscle cells or nerve fibres, owing to the fact that it is readily dissociated (see Part I) and also to the unfavourable conditions for absorption.

III. PATHOLOGICAL CHANGES.

(i) *Macroscopical.*

As has been stated, rabbits usually assumed a position of orthotonos before death occurred and the cadaver retained this position. In cats, this characteristic was not noted.

Rigor mortis took place normally. No typical external appearances were present after death.

No changes have been seen in the heart after poisoning with nickel carbonyl. In one case, emphysema pericardii was present.

The Lungs.

The changes met with in the lungs consisted of congestion, oedema, haemorrhage and compensatory emphysema.

When death occurred during the first 24 hours, the hyperaemia was often the only change present. In the majority of cases, however, there was fairly well marked oedema and at times a few punctate haemorrhages were seen on the pleural surface and on section. Parenchymatous haemorrhages of considerable size were rare at this stage.

When death occurred during the second day, the hyperaemia was intense. The lungs were freely bathed in a blood-stained fluid. Numerous small and medium sized haemorrhages, not only on the pleural surface, but also pervading the whole organ were common. Large haemorrhages were less frequent. In some cats, patches of consolidation were seen at this stage.

When death took place during the third day, small patches of more or less complete consolidation were usually present. These patches were of dark mulberry colour and exuded blood when squeezed. The haemorrhages which affected considerable areas of lung also gave the appearance of consolidation. They were large or small and were usually scattered over the whole organ, save in those situations in

which emphysema existed. The emphysema was best seen at the free edges of the lobes and a characteristic mosaic-like appearance was seen on the pleural surface. Oedema was rarely clearly seen by the naked eye at this stage on account of the other changes which obscured it.

When death took place during the 4th or 5th day, the infiltration with blood-stained fluid, the consolidation and haemorrhagic foci had reached their maximum. A few fresh haemorrhages were found in a few cases but the majority were of older standing. Whole lobes or even whole organs presented an almost uniform mulberry colour and had the consistence of liver. The tissue sank in water. On section, a frothy blood-stained fluid could be expressed.

Pleural effusion was found in one case.

The Bronchial Glands.

In cats, the bronchial glands were seen to undergo changes in keeping with those in the lungs. At first there were some swelling and redness. The colour then became darker and small haemorrhages were seen. Dark specks were seen in the substance after about 36 hours; these specks sometimes had a greenish tinge. Later, the haemorrhages became more intense.

Trachea, Larynx and Bronchi.

In practically all the cases, the mucous membrane of the upper air passages was markedly injected for the first few hours after the inhalation. Haemorrhages were sometimes seen early, but more often they occurred at a later stage. The passages often contained a viscid greenish fluid or else a clear frothy or blood-stained fluid.

Liver, Pancreas, Spleen, Kidneys, Bladder and Genital Organs.

Changes in these organs were seen only on rare occasions. In a few cases, haemorrhages were met with in the kidneys, spleen, pancreas, ovaries, testes and uterus.

Intestines and Stomach.

Haemorrhages were seen at times in the mucous membrane of the stomach and very rarely in that of the intestines. Congestion and ulceration were also met with exceptionally.

Adrenals.

Changes in the adrenals were met with in the majority of the cases. These were first seen after the 2nd day. Small localized or large diffuse haemorrhages were common and general swelling and redness of the organs usually accompanied them. At times there was only congestion. The haemorrhages were usually situated in the medulla of the adrenals.

Brain.

Changes were commonly met with in the brain and were sometimes extensive. The meninges were generally suffused with blood, the vessels were dilated and at times the membranes thickened. In rare cases, some meningeal haemorrhages were seen. The changes were met with from the 2nd day onward and were the more intense the later death took place.

As a rule, the white matter of the brain was the seat of haemorrhages. These were generally minute. They were seen in the *optic thalami*, *corpus striatum*, internal capsule and *pons varolii*, as well as in other situations. In the medulla and cerebellum, haemorrhages were also seen in a number of cases. Simple congestion was met with frequently. Extensive haemorrhages in the brain of rabbits, cats and dogs, like those observed in the brain of one of the human subjects, who died of plant gas poisoning at the Nickel Works, have not been met with.

The Spinal Cord.

In a few cases, in which the spinal cord was examined, no pathological changes were seen.

The following table shows the frequency of haemorrhages met with in the various organs.

TABLE II.

Showing the frequency of haemorrhages in the various organs.

	Cats (31)	Rabbits (93)
	100 %	100 %
Lungs	100	100
Liver	6.4	4.3
Spleen	0	2.1
Pancreas	3.2	1.0
Kidneys	0	6.4
Adrenals	83.8	51.6
Stomach	0	9.6
Genital organs	0	5.3
Brain	74.1	37.6

(ii) *Microscopical.*

It is only necessary to describe the microscopical appearances of the lungs, bronchial glands, adrenals and brain, as the changes met with in other organs were rare and corresponded exactly to the macroscopical appearances.

The organs were fixed and hardened in potassium bichromate and acetic acid or Flemming's osmic acid fluid followed by alcohol. Brains were either put through alcohol, bichromate of potassium, formaline or Muller's solution or else fixed by Marchi's method.

The Lungs.

Oedema is the most striking of the early changes. The specimens show a diffuse homogeneous material, which stains more or less deeply with eosin. The fluid is usually situated in the pulmonary tissue, lying like a stroma in the network of the interalveolar tissue. At times, however, it pervades the spaces and may be seen either in the alveoli or even in the bronchioli and bronchi (see Plate VII, Figs. 1 and 2). Often the fluid contains a few blood corpuscles.

Haemorrhages vary considerably in extent. They may be so extensive that the lung structure is quite obliterated. The blood cells are first poured out into the interalveolar tissue and pass into the air vesicles and passages later. In early cases, diapedesis may be marked. The endothelial cells of the capillaries at first appear swollen, then degenerative changes are seen, then the wall gives way at one spot and the blood issues out, gradually enlarging the defect produced in the wall.

Leucocytosis in the vicinity of the capillaries is often extensive in limited areas, and after a time, peribronchitic small cell infiltration is seen. The alveoli and smallest bronchioli become more or less filled with blood cells and the appearance when whole lobules are affected only differs from that of typical broncho-pneumonia by the occasional presence of the homogeneous fluid and haemorrhagic patches. In advanced cases, there is a typical inflammatory process, identical to that of pneumonia, including the polynuclear exudate in the alveoli, pus cells in the bronchi and well marked desquamation of the endothelium. At times, one meets with some proliferation of the bronchial epithelium. In the earlier stages, the polymorphonuclear cells are usually absent and the infiltration is then limited to small round cells.

The Bronchial Glands.

Considerable congestion, small cell infiltration and haemorrhages may be seen quite early. The lymphoid tissue appears at first to be but little affected, since the leucocytosis and haemorrhages are not extensive. Later on more extensive haemorrhages may occur. In some situations minute intracellularly placed granules of dark colour may be seen. At times, the endothelial cells take up red blood corpuscles.

The Adrenals.

The changes met with correspond to those in the lungs. In the earliest stages, the endothelial cells of the capillaries undergo degeneration, then they rupture and the blood cells are poured out into the surrounding tissue. Later on the parenchymatous cells may degenerate secondarily (see Plate VIII, Fig. 5). During the early stages, leucocytosis may be seen in the neighbourhood of the capillaries. Primary changes in the parenchymatous cells cannot be detected, but the fact that haemorrhages frequently obscure the early changes must be taken into account before one can definitely exclude the possibility of their existence.

The Brain.

In this organ, the changes are like those met with in the lungs. The earliest changes seen are found in the walls of the capillaries and small blood vessels. The endothelial cells are swollen, the nuclei are badly stained and the outlines of the cells are not well differentiated. Many of the cells are misshapen. When fixed by osmic acid, fat globules are seen both below the endothelial layer and in the endothelial cells themselves (see Plate VIII, Fig. 6). In a few situations, fat is seen in the lumen of the vessel, in cells lying free. These cells appear to be like endothelial cells and it is probable that they had been cast off.

After the cells have become sufficiently affected, they are no longer capable of withstanding the pressure of the blood and thus the vessel gives way, at first by the failure of one or two cells and later by a number of contiguous cells becoming destroyed or pushed aside. The blood escapes from the vessel in comparatively narrow tracks and the cells are found closely packed together (see Plate VIII, Fig. 7). A considerable amount of leucocytosis is seen in the neighbourhood of

the affected capillaries, especially before the rupture takes place. The leucocytes are frequently grouped around nerve ganglion cells and appear to be connected with the changes which take place in the latter, even before a haemorrhage takes place. At first, the Nissl's granules become indistinct and stain weakly. Later on, they become more and more ill defined until they disappear and the contents of the cell appears to be homogeneous (see Plate VIII, Fig. 8). During the later stages, the processes of the ganglion cells can no longer be demonstrated. In the majority of cases, the chromatolysis does not become complete.

Secondary degeneration of the nerve fibres is not often seen in cats and never in rabbits, since the time between the inhalation and death is too short for such processes to be marked. Slight Marchi degeneration was seen in the case of a cat, in which repeated sub-lethal doses of nickel carbonyl were given, with the effect that the cat lived for 22 days, during which time it was continually under the influence of nickel.

IV. ATTEMPT TO TRACE THE NICKEL THROUGH THE ORGANISM.

(i) *Methods of detection.*

The detection of nickel in the various tissues was carried out in two ways. Firstly the organs were ashed and the nickel was determined quantitatively by the dimethyl glyoxime method, described by Harden and myself (1906)¹. Secondly the nickel was recognized by micro-chemical means. The first method was necessary in order to obtain information with regard to the passage of the nickel and the rate of its passage, while the second method was found only to be available during the earliest stages in the lungs, before the nickel had entered into complex combination.

Considerable difficulty was experienced in working out a satisfactory method of micro-chemical detection of nickel in the tissues. Two reactions were found to be applicable. The first of these is the dimethyl glyoxime reaction, but this has a great disadvantage in that the compound first forms in solution and subsequently crystallizes out. The appearance of the sections, however, was very characteristic, when the preparation was carried out as follows. A rabbit was exposed to a high concentration of nickel carbonyl vapour and on being removed

¹ Harden and Armit. *Proc. Roy. Soc.*, 1906.

from the poison chamber was at once killed. The lungs were removed and portions were cut fresh by the freezing microtome. The sections were first floated out on a weak solution of sodium triphosphate, to prevent a solution of the nickel, then they were transferred to a solution of dimethyl glyoxime in methyl alcohol, to which a few drops of ammonia had been added and allowed to remain in this solution for several minutes. They were then rapidly passed through water containing a few drops of ammonia and spread out on slides. The specimens were examined either unstained or faintly stained in methylene blue. The nickel appeared as a diffuse red staining of the tissue, which however paled rapidly and from which red needle shaped crystals separated out. The position of these crystals was no indication of the position of the nickel. The other method showed that the temporary appearances observed by means of the first method actually corresponds to the position of the nickel.

Another method for the detection of nickel in the tissues was employed. A rabbit was poisoned by inhalation of nickel carbonyl in air for 25 minutes. After an hour during which the nickel carbonyl would be entirely dissociated, it was killed. The lungs were removed and blown up several times with sulphuretted hydrogen for about thirty minutes. They were then blown up with an indifferent gas (carbon dioxide was used), which first passed through a tube immersed in boiling water. The lungs were kept during this stage in a dry vessel at a temperature of 90° C. After the lungs had been heated in this way for three hours, portions were removed and cut by the freezing microtome. The sections were floated out on water, passed through spirit and again floated out on water. They were then spread out on slides and stained with haematoxylin. The nickel was seen as a brownish staining of the tissues. On examining the affected parts with a high power, very minute granules of a brownish colour could be detected. A similar appearance was seen when a solution of the dissociation product of nickel carbonyl in serum (see Part I of this paper) was treated with sulphuretted hydrogen and the serum coagulated by heat was examined under the microscope. The solution of the nickel sulphide in serum is a dark brown almost clear solution, but when seen under a high magnification, it is found to contain minute granules of dark colour.

(ii) *Evidence of what takes place in the lungs.*

Fate of nickel carbonyl when inhaled in the lungs.

In tracing the nickel carbonyl through the organism, it is necessary to enquire what happens at the site of absorption. It is therefore desirable to consider the immediate result both to the carbonyl and to the lungs when the vapour is inhaled. It has already been shown in Part I of this paper, that nickel carbonyl vapour when placed in contact with air and moisture at the temperature of the animal body dissociates rapidly. Evidence that dissociation takes place in the air passages is found in the deposition of a nickel containing substance on the surface of the mucous membranes. In order to follow the process, it is necessary to consider what amount of nickel is available for absorption and also in what way and with what rapidity it is absorbed by the blood.

Direct measurement of the quantity of nickel carbonyl dissociated could not be carried out, on account of the difficulty presented to accurate analysis by the small differences in the volume per centage of the already dilute gas mixture before and after passing through the poison chamber.

Indirect evidence is however obtainable. The maximum amount of nickel carbonyl available is calculated by determining the amount of air mixture which the animal breathes during the experiment. As has been stated, the inhalation of 0.018 volume per cent. of nickel carbonyl vapour in air for 50.5 minutes suffices to kill rabbits. A rabbit weighing 2 kilograms takes in about 600 c.cm. of air per minute under normal conditions. It would therefore breathe about 32 litres during the inhalation. The total amount of nickel carbonyl vapour breathed would therefore be about 5.9 c.cm or 0.045 gram. This would contain about 0.0155 gram of nickel. The total quantity of nickel carbonyl entering the lungs would correspond to about $7\frac{1}{2}$ mgrs. of nickel per kilogram body weight.

The animal would not absorb all the vapour which is contained in the air entering the respiratory passages, and evidence that some of the vapour is not absorbed is found in the fact that the expired air of an animal immediately after the inhalation contains recognizable quantities of nickel carbonyl. The amount mentioned above must therefore be regarded as a store from which the animal will abstract a fraction, which will serve as a lethal dose.

In cats, the inhalation of 0.04 volume per cent. of nickel carbonyl in air for 75.5 minutes proved sufficient to kill. A cat weighing 3 kilograms breathes about 800 c.cm. per minute. This would correspond to about $60\frac{1}{2}$ litres during the inhalation. This is equivalent to about 24.3 c.cm. or 0.184 gram of nickel carbonyl or roughly 64 mgrs. of nickel. The available amount of nickel carbonyl expressed as nickel would therefore not exceed 21 mgrs. per kilogram body weight.

In the case of a cat which was affected with an old pulmonary lesion, probably of tubercular origin, and which died at the end of the inhalation before it could be removed from the poison chamber, 32 mgrs. of nickel were recovered from the lungs after ashing, while the blood was found to be free from nickel. The cat weighed 3800 grams, so that 8.4 mgrs. of nickel per kilogram body weight was found. In other cases, the amount recovered from the lungs was less than this amount, since absorption from the lungs takes place early. The average amount recovered was about 10 mgrs. The amount found in the blood of cats immediately after inhalation varied up to 3.75 mgrs. per 100 grams of blood. Thus in cats weighing up to 3 kilograms, from 20 to 22 mgrs. of nickel has been accounted for in the lungs and blood, while small quantities would probably be present in other tissues (e.g. the bronchial glands usually contain small quantities early). Making some allowance for this and having regard to the result of the experiment in which 32 mgrs. was recovered, it may be assumed that after inhaling 0.04 volume per cent. of nickel carbonyl vapour in air for $75\frac{1}{2}$ minutes, cats are able to dissociate an amount of nickel carbonyl representing not less than $8\frac{1}{2}$ mgrs. of nickel per kilogram body weight. This would correspond to between $\frac{1}{3}$ and $\frac{1}{2}$ of the total available amount. In rabbits, up to $3\frac{1}{2}$ mgrs. of nickel per kilogram body weight has been accounted for in the same way immediately after inhalation. The absorption of about one half of the inspired quantity of a toxic gas has been met with in the case of carbon monoxide (Haldane, 1895).

It has already been stated that from the earliest stage of nickel carbonyl poisoning, the lungs undergo changes. It is uncertain whether the vapour as such acts as an irritant. Nickel carbonyl is dissociated into carbon monoxide and a nickel containing product which is probably a hydrated sub-carbonate of nickel (see Part I, p. 541 et seq.). The quantity of the carbon monoxide is too small to produce any appreciable effect, since the amount of carbon monoxide liberated from the nickel carbonyl corresponding to $8\frac{1}{2}$ mgrs. of nickel would be less than $9\frac{1}{2}$ c.cm., and this small amount would be available

for absorption during the 75½ minutes (see also Part I, p. 528). The nickel containing substance is gradually dissolved by the tissue fluids and the dissolved nickel acts locally upon the peribronchial and alveolar tissue as well as on the endothelial cells of the capillaries producing the changes which have already been described. The production of a serous effusion or oedema hastens the solution of the deposited nickel compound. At first, the nickel containing product of dissociation is simply dissolved, without any alteration of its chemical constitution, but after the course of some hours, it becomes acted on by the proteid material and a more complex nickel combination results. The evidence of this is found in the fact that at first the red compound forms with dimethyl glyoxime readily, but later on this combination only takes place slowly, and after about 24 to 36 hours although there is still nickel in the lungs, as demonstrated by analysis after ashing, neither the dimethyl glyoxime compound nor the sulphide can be formed.

At times, the absorption takes place very rapidly and in one case of a small rabbit, less than 1 mgr. of nickel was found in the lungs immediately after poisoning. The process of absorption however takes some time to complete and 3 mgrs. has been found 24 hours and 1 mgr. 48 hours after the inhalation in rabbits. From this time onwards only small traces could be detected and although 0·035 mgr. was found in one rabbit after 96 hours, the nickel was usually completely removed after about 2½ days.

The microscopical appearances assist in determining the way in which the nickel is taken up. The free surfaces and the tissue immediately surrounding the surface of the bronchi, bronchioli and alveoli show red or brownish staining when the sections have been treated by the dimethyl glyoxime or sulphide methods. The study of the dissociation of nickel carbonyl described in Part I has shown that under the conditions of temperature, moisture and carbon dioxide obtaining in the lungs, the dissociation product is formed, which is slightly soluble in water and in solutions of phosphates. It is thus clear that the nickel subcarbonate is deposited on the respiratory mucous surfaces and is gradually dissolved from this situation by the tissue fluids. This accounts for the intense staining at the free edges and the diffuse staining in the neighbouring tissues.

(iii) *The absorption of the nickel by the blood.*

Evidence of the absorption of the nickel deposited on the respiratory surface and dissolved from this situation by the tissue fluids may

be obtained from (1) the presence of nickel in the blood during the early stages, (2) the gradual disappearance of the nickel from the lungs, (3) the appearance of nickel in other organs, and (4) the appearances of the micro-chemical tests in the lungs during the early stages.

(a) The fate of nickel carbonyl in artificial perfusion experiments.

In following out the fate of nickel carbonyl after inhalation, a short series of experiments with artificial perfusion of the lungs was carried out. It was found that when cats' lungs were employed, the experiments were unsatisfactory, as the tissues became oedematous early and thus terminated the experiment before sufficient time had elapsed. Dogs' lungs were therefore used.

The experiments were carried out with an apparatus devised by Martin and Embley (1905)¹. The lungs were removed and the respiration was maintained by inflating them rhythmically with air containing varying amounts of nickel carbonyl vapour. The animal's own blood defibrinated and kept at 37° C. was used, which was circulated through the lungs. Samples of blood were taken from time to time, and ashed to determine the quantity of nickel which had been taken up. At the end of the experiments, the lungs were also ashed and examined quantitatively for nickel.

The results of two experiments conducted in this way were as follows:

TABLE III.

	Duration in mins.	Amount of air used (litres)	Amount of Ni (CO) ₄ used (grams)	Vol. % of Ni (CO) ₄ vapour	Blood (grams)	Mgrs. Ni per 100 grams blood	Mgrs. Ni in lungs
1.	90	891	0.9436	0.014	300	(a) 0.05 (b) 0.019 (c) 0.037	3.00
2.	50	450	8.8188	0.251	250	(a) 1.75 (b) 1.91	25.87

From these figures it would appear that although the greater part of the nickel is left in the lungs, some of it is taken up by the blood. The amount in the blood is larger the more concentrated the nickel carbonyl vapour. In the first experiment about 0.1 mgr. of nickel appeared in the total quantity of blood used and in the second

¹ Martin and Embley. "The action of anaesthetic quantities of chloroform upon the blood vessels of the bowel and kidney; with an account of an artificial circulation apparatus." *Journ. Phys.* xxxii. p. 147. 1905.

experiment about 4·5 mgrs. These quantities are extremely small as compared with the 3 mgrs. and 25·8 mgrs. found respectively in the lungs.

The first sample taken in the second experiment was found to contain carbon monoxide corresponding to 21·4 % of the quantity necessary to saturate. This is quite consistent with what has already been said on the subject of carbon monoxide absorption (see Part I, p. 529), since the concentration of the nickel carbonyl vapour was considerably greater than was employed in the inhalation experiments on animals.

The conditions of these experiments are not exactly similar to those of inhalation by a living animal. The surface of the lung was considerably cooler and the tissues were in a dying condition. The experiments, however, show that dissociation of nickel carbonyl takes place rapidly in the lungs at body temperature and that the nickel is at first deposited in the lungs to be gradually dissolved and carried away by the blood. In the experiments it is possible that some nickel carbonyl went into solution in the blood but it would be rapidly dissociated before it had time to leave the blood again. The fact that the lungs became oedematous and thus terminated the experiments when high concentrations were used sooner than when lower concentrations were used points to a direct toxic action of the nickel on the tissue.

(b) Rate of absorption of nickel from the lung.

The blood, as has already been shown, contains varying quantities of nickel during the early stages. At times no nickel was recovered after ashing small samples within the first day, but as a rule, especially when 50 grams were ashed, easily detectable amounts were regained. The rate of the removal of nickel from the pulmonary tissue will depend on the conditions of the circulation obtaining for the time. The presence of a finely divided nickel compound on the mucous surface produces an inflammatory reaction. The leucocytes and inflammatory cells increase the density of the organs. A serous exudation is thrown out and the air passages become more and more encroached upon, and as a result parts of the respiratory surface become obliterated. It has further been shown that nickel coming into contact with the capillary walls causes changes of a fatty degeneration (this was best demonstrated in the vessels of the brain). The cells give way and one of the results of the haemorrhages which ensue is that the organs become still denser. The circulation through the lungs is thus slowed down by the resistance of the solid organ and by the mechanical interference to the circulation brought about by the rupture of the capillaries and consequent coagulation. Owing to the slowing of the blood stream, the nickel finds it

increasingly difficult to get out, and as a result nickel can still be found in the lungs at the end of 60 hours or at times even later.

The quantity of nickel during the second and third day which is present in the blood at any given time must be extremely small, and it is therefore not surprising that none was detected during the second day in any of the analyses. As will be mentioned later, there is reason to believe that nickel is rapidly claimed by other tissues, and this would therefore lessen the quantity present in the blood at any given time. Little by little however the nickel is washed out of the lungs into the blood stream. A certain amount finds its way into the bronchial glands through the lymphatic channels. It can therefore be said that the amount taken up by the blood and available for other tissues at any given time is inversely proportionate to the acuteness of the onset of the pneumonic processes. The more slowly the consolidation of the lungs takes place, the larger will be the amount of nickel which enters the circulation at any one time.

(iv) *Deposition of nickel in the tissues and organs.*

It has been stated that evidence of the absorption of the nickel was obtained by the appearance of nickel in other organs. The various organs and tissues were analysed for nickel at the various stages of the poisoning, but no nickel was detected at any time in the liver, spleen, pancreas, muscles or bile. A very small trace was found on one occasion in the heart immediately after the inhalation, but this was probably due to the accidental inclusion of some blood clot. Nickel was found in the bronchial glands within a short time after poisoning. In the kidneys, nickel was only found at a late stage. This fact will be referred to when the subject of the elimination of the nickel is discussed. The adrenals contained nickel from the 36th hour after inhalation onward. As a rule, the quantity was very small and at the later stages, it did not exceed 0.02 or 0.03 mgr. It was also found in the brain regularly after the end of the first day until the end of the fourth day. A table is appended which shows the position of the nickel during the successive stages of the poisoning.

Evidence of selective absorption by certain tissues.

The results of these analyses point to a selection on the part of the adrenals and brain. The nickel must be available for all the organs, but save for an occasional lesion in other organs, there is no evidence of the presence of the toxic metal in them. The blood yields up its nickel

to the adrenals, and brain during the course of the second, third and fourth days. A certain amount of nickel is found in the bronchial glands early, but this is more probably due to the absorption of the nickel either in solution or even in solid form in the bodies of leucocytes through the lymphatic channels. The presence of nickel in the kidneys and the occurrence of pathological lesions in these organs may be neglected for the time being. It must also be pointed out that the lesions

TABLE IV.

Showing the localisation of the nickel in the tissues and organs at varying times.

	0—12 hrs.	12—24 hrs.	24—36 hrs.	36—48 hrs.	48—60 hrs.	60—70 hrs.	Later
Lungs	+++	+++	++	++	+	0	0
Bronchial glands	+	++	++	++	...	0	...
Stomach	...	...	...	...	0	0	0
Adrenals	...	0	0	++	++	++	+
Brain	0	×	×	++	+++	++	+
Kidneys	0	...	0	...	+	++	+
Blood	++	+	0	0	++	++	+
Milk	...	...	...	...	++	...	...
Faeces	...	...	0	...	++	++	++
Urine	0	0	+	++	++	+++	++
Bronchial secretion	++	++	++	...	...	...	...

+ indicates present at times. ++ indicates present.

+++ indicates present in comparatively large quantities.

0 indicates absent. × indicates present on rare occasions.

found in the organs are practically limited to the lungs, adrenals and brain, both in nickel carbonyl poisoning and also, as will be shown later, in poisoning by nickel and its other compounds. The urine contained nickel from the 24th hour till the fourth day or later, and this would further support the contention that the nickel does not remain long in the organs but passes through them, producing damage during a short stay. One is therefore unable to present evidence typical of selective absorption, namely a definite proportion between the quantity of the nickel and the mass of the organ. The maximum amount of nickel found in the brain was about 0.01 % of the weight of the organ and 0.006 % of the weight of the organ in the adrenals. In spite of this, however, the failure of detecting any nickel in the liver and other organs and the localisation of the pathological lesions must be regarded as evidence of selection on the part of the adrenals and brain.

(v) *Elimination.*

Nickel is excreted in the urine and faeces. On one occasion the milk of a lactating cat was examined and was found to contain nickel on the third day. No nickel was found in the stomach contents, save once in the case of a rabbit in which death occurred early. It is however probable that some bronchial secretion had been swallowed, as this contained nickel.

About 75% of the nickel excreted is found in the urine in rabbits, while in cats over one half of the total quantity is got rid of in this way. The rate of elimination through the kidneys was found to be very irregular, the maximum however being usually reached on the fourth to the fifth day. When the urine contained comparatively large quantities of nickel, some metal was also found in the kidneys. The changes found in these organs were probably produced at this stage, since no congestion or haemorrhage was met with when death took place before the end of 60 hours after the inhalation.

The quantities of nickel in the faeces also reached a maximum about the fourth or fifth day. Diarrhoea usually set in about this period and it was found that the amount of nickel was always greater when the stools were fluid. The faeces then assumed a darkish colour. The nickel cannot be extracted by the action of water or dilute acids and it is probably present in the form of sulphide.

At times death took place after all the nickel had been excreted.

The nickel in the urine appeared to be in a condition of complex combination. Urine containing nickel does not give the dimethyl glyoxime or the sulphide reactions, but after boiling with concentrated nitric acid and neutralising with ammonia both reactions can be obtained. The nickel cannot be precipitated by heat. The urine was subjected to dialysis. After 24 hours, the process was incomplete. In one case, 90 c.cm. of urine was dialysed with 400 c.cm. of water. At the end of 24 hours, 30% had passed through the membrane, while nearly 60% was left inside the skin, the remainder having been absorbed into the membrane. This subject will be again referred to when the excretion of metal in cobalt poisoning is discussed.

An attempt was made to remove a possible organic compound of nickel from the tissues by means of certain solvents (aether, alcohol, saline fluid and water). While aether failed to extract any nickel, the other solvents removed part of the metal. The amounts extracted were very irregular and in no case was the whole amount extracted.

V. POISONING WITH NICKEL AND NICKEL SALTS.

Since nickel carbonyl poisoning is actually poisoning by nickel, in which the metal is applied to the enormous area of the respiratory surface, the symptoms and pathological changes met with in nickel poisonings should correspond with those met with in nickel carbonyl poisoning, allowance being made for the mode of entry.

Laborde and Riche (1888) found that when the soluble salts of nickel are given by mouth, vomiting is produced, but even when 3 grams of nickel sulphate is used, death does not follow. A. Riche (1888) however met with late death after feeding with food impregnated with nickel sulphate in dogs. Death has been seen when the sulphate was injected subcutaneously and intravenously by these two observers. The lethal dose of nickel sulphate applied in this way was found to be 0.0655 gram for rabbits, while 0.378 gram was required for dogs.

Nickel sulphate however is an extremely unsuitable salt for toxicological experiments, as it coagulates albumin and whether injected subcutaneously or intravenously, it is only absorbed very slowly and incompletely owing to the small surface for absorption of the coagulated mass. According to Stuart (1884) the lethal dose of nickel (given as NiO) for rabbits and cats is about 8 mgrs. per kilogram body weight, while for dogs it is higher.

The nickel compounds dealt with in the present investigation are the carbonate, the tri-phosphate, the acid phosphate, the pyro-phosphate, the hydrate, the sulphate, the chloride, the acetate, the ferro-cyanide, the sulphide, the tartrate and the potassium albuminate. Metallic nickel in a state of very fine division was also used. The animals experimented on were guinea-pigs, rabbits and cats. The various preparations were introduced by subcutaneous, intravenous and intraperitoneal injection.

1. Subcutaneous injection.

0.0023 gram of nickel in the form of sulphate, phosphate, ferro-cyanide and chloride killed guinea-pigs. The same dose of nickel in the form of acetate also killed. This corresponds to about $6\frac{1}{2}$ mgrs. of nickel per kilogram body weight. 0.008 gram of nickel as carbonate was required to kill guinea-pigs. The lethal dose by this method for rabbits with the soluble salts was found to be between 7 and 8 mgrs. per kilogram body weight. Cats died after the injection of 0.23 to 0.03 gram of nickel as nickel sulphate. The lethal dose for cats per kilo-

gram body weight thus appears to be from 9 to 16 mgrs. calculated as nickel.

2. Intravenous injections.

A few experiments were conducted with intravenous injection but the method was discarded when it was found that the soluble salts, such as the sulphate and chloride, and the insoluble salts, such as the hydrate and carbonate, mostly blocked up a small vessel and unless injected in comparatively large quantities or in minimal quantities in several situations, rarely produced symptoms.

3. Intraperitoneal injection.

The insoluble salts were freshly precipitated and injected with aseptic precautions into the peritoneal cavity. Especial attention was paid to the behaviour of the physically indifferent salts, such as the hydrate, carbonate and phosphates. Guinea-pigs died after receiving 5 mgrs. per kilogram body weight, but after death some unabsorbed salt was found in the peritoneum. When larger doses of these salts were used (e.g. 12 mgrs. of nickel) the unabsorbed material could be recovered to a great extent and estimated after dissolving in acetic acid. From these estimations, it would appear that death follows after the absorption of between 3 and 4 mgrs. of nickel per kilogram body weight.

The tartrate was further used to determine the lethal dose, and it was found that rabbits died after the injection of 8 mgrs. of nickel per kilogram body weight. It was noted however that the absorption of the tartrate is more complicated than that of the salts containing free nickel ions, and from the experiments conducted with the acetate, hydrate and phosphate, it appears that the minimum lethal dose for rabbits is less than 7 mgrs. of nickel.

Nickel sulphide was injected into the peritoneal cavity. 0.025 mgr. (calculated as nickel) led to death in two out of three guinea-pigs. After death a large quantity of black material was found in part free and in part adherent to the surface of the peritoneum. Around the adherent particles, a distinct greenish colour was seen at the periphery. The tissue was removed and treated with acetic acid. Nickel was found in the filtrate, showing that the sulphide had been reduced and that the nickel had combined with oxygen or carbon dioxide.

Metallic nickel was prepared from the hydrate or from the oxalate by drying and reducing in hydrogen at about 260° to 280° C. and sealing up the tubes in which the reduction had been carried out. The metal prepared in this way existed in a state of very fine division and was pyrophoric (i.e. took fire spontaneously when exposed to oxygen). Some

of the samples worked with were less finely divided than others. The end of the tube was broken off and a little well boiled physiological saline solution was immediately run into the tube, before the hydrogen in the tube had time to diffuse. The metal was then suspended in the fluid by rapid rotatory movements and sucked up into a glass syringe, without any contact with air. The suspension was injected into the peritoneal cavity of guinea-pigs and rabbits.

The exact dosage was difficult to determine owing to two circumstances. Firstly a little material was always left in the tube, syringe and needle, and secondly after death a varying amount chiefly comprising the coarser particles and clumps which had formed was found unabsorbed. In guinea-pigs, the quantity injected varied between 12 and 28 mgrs. and all the animals thus treated died save a few in which metal which was not very finely divided was injected. 28 mgrs. of nickel in a state of only moderately fine division failed to kill a rabbit, while 30 mgrs. of the same sample killed a second rabbit. Post mortem, relatively large quantities of black material clumped in patches were found in the peritoneum. In every case it was evident that only a part of the nickel injected had been absorbed.

The absorption of the nickel was studied further in these cases. The mesentery which contained small macroscopically visible deposits of black material was stretched on cork discs, fixed, transferred to cover glasses, stained and mounted. In these one could see that the very minutest particles had been taken up by the polynuclear cells and to a less extent by the endothelial cells and by some of the red blood corpuscles. Phagocytosis of the red cell was also seen in situations where the red cells had taken up nickel particles. Nickel granules were further seen in the walls of the lymph spaces, lying free. No granules either intracellularly or extracellularly placed were seen in the blood vessels. The taking up of the nickel appeared to be most extensive about 24 hours after the injection but even as late as 70 hours it could still be seen.

Experiments were further carried out *in vitro*. It was found that pyrophoric nickel, nickel sesquioxide and nickel sulphide in fine state of division are readily taken up by the polymorphonuclear cells.

Symptoms and pathological changes in nickel poisoning.

Guinea-pigs poisoned by nickel and nickel salts all exhibited the same symptoms and post mortem changes. The former consisted in dyspnoea, paresis, at times paralysis and later diarrhoea, cyanosis and convulsions. The lungs showed haemorrhages, compensatory emphysema and some oedema and the adrenals showed haemorrhages.

Rabbits exhibited symptoms like those of nickel carbonyl poisoning, with the exception of the early dyspnoea and giddiness. The respiratory rate became increased after a few hours, the temperature began to fall, the appetite was lost and the animal became dull and listless. These symptoms increased steadily until the dyspnoea became intense and was accompanied by cyanosis. Paresis was only seen in a few cases. Diarrhoea was usually seen after about 24 to 36 hours and death followed in the course of from a few hours to three or four days. The temperature sank to a low level before death.

In cats, the subcutaneous application of soluble salts produced characteristic symptoms. At first general illness without any definite signs was seen, later the appetite was lost, the weight fell and vomiting and diarrhoea set in. Paresis or even paralysis was noted and the gait became ataxic. Tremors of the anterior extremities and of the head occurred. The respiratory rate increased within a short time. Death occurred within the first 12 hours when large doses were injected, while when smaller doses were employed, it was delayed for some days but not exceeding eight days.

Post mortem the lungs, adrenals and brain showed similar changes to those seen after nickel carbonyl poisoning. The pulmonary changes were not so extensive or intense as those met with in nickel carbonyl poisoning, presumably because the whole quantity of the metal does not pass through the lungs as it does when the vapour is inhaled. When death took place late, large areas of lung tissue were affected, and it was impossible to distinguish the sections from those of nickel carbonyl poisoning. The kidneys were more frequently affected in poisoning with the soluble salts of nickel especially when these were given intravenously.

VI. COMPARISON WITH IRON AND COBALT POISONING.

(i) *Iron carbonyl poisoning.*

As it is proposed to record the results of experiments conducted with iron carbonyl, pyrophoric iron and iron salts as well as with cobalt and its salts elsewhere, only brief reference to these experiments will be made in this place. It was found that 0.025 volume per cent. of iron carbonyl in air inhaled for 45.5 minutes (after deducting 14.5 minutes, see p. 567) killed rabbits. This yields a maximum quantity of iron of about 17 mgrs. It thus appears that iron carbonyl is less toxic than

nickel carbonyl. On the other hand, death takes place slightly more rapidly, the average length of life after poisoning with minimal quantities being 40·4 hours as against 69·3 hours after poisoning with nickel carbonyl.

The symptoms of iron carbonyl poisoning showed considerable similarity with those of nickel carbonyl. Beside the respiratory distress, dyspnoea, cyanosis, loss of weight and fall in temperature, coarse tremors were noted and also spastic gait and paresis of the hind extremities. The heart beat was frequently irregular and intermittency of the beat was noted. The physical signs of consolidation of the lungs were detected earlier than in nickel carbonyl poisoning. Diarrhoea was not present in any of the cases, and when recovery followed the poisoning, a peculiarity of the gait persisted for some time.

The pathological changes met with were of the same type as those of nickel carbonyl poisoning, but all the organs were affected without any preference. In the lungs, the pneumonic process was often intense and not infrequently enormous haemorrhagic infiltration was seen (see Plate VII, Fig. 3). No essential difference between the effects of the two carbonyls upon the lungs could be ascertained. The spleen, kidneys, stomach, intestines and other organs mostly showed haemorrhages and degeneration of parenchymatous cells, while the adrenals were only occasionally affected. The heart was generally dilated and haemorrhages were found in the walls. The brain also showed haemorrhages similar to those of nickel carbonyl poisoning.

The faeces were rarely dark in colour, save when the symptoms lasted for a long time, and diarrhoea was never met with. Iron was found in the faeces and urine. In the urine, it existed partly as a ferric and partly as a ferrous compound.

Comparative experiments with iron and iron salts were carried out in the same way as was done with nickel and nickel salts. The symptoms and pathological changes of iron and iron salt poisoning corresponded to those of iron carbonyl poisoning. The lethal dose of iron when given by intraperitoneal injection proved to be about 20 mgrs. per kilogram body weight. It could be shown that the ferric condition of the metal was retained in the excretion when a ferric compound was used, and the ferrous condition when a ferrous salt was introduced. Micro-chemical examination however showed that in the tissues, free iron ions were present, even if the compound introduced was in a condition of complex combination.

Cobalt was also studied in a similar manner. From these experi

ments, it could be shown that no essential difference between poisoning with nickel and poisoning with cobalt exists. On Plate VII, Fig. 4 the changes met with in the lungs after subcutaneous injection with cobalt chloride are shown, and from this it will be seen that all the characteristics of nickel poisoning are present. The only means of determining between the two conditions is by chemical analysis and by the appearance of the urine. The lethal dose of cobalt is higher than that of nickel, rabbits dying after the absorption of from 15 to 18 mgrs. per kilogram body weight, when applied intraperitoneally.

The urine of cobalt poisoning presents a peculiar colour. This was first described by Stuart. The first traces of the colour was seen within 15 minutes of the intraperitoneal injection of the tartrate of cobalt. As a rule, the colour attained its maximum during the course of the second day, but traces were seen during the following two or three days. The colour is a characteristic brown one. As this colour proved to be an easy index of the presence of cobalt in the urine, urine containing it was carefully studied. It was found that the quantity of cobalt varied in proportion with the intensity of the colour. When subjected to dialysis, the cobalt like the nickel passed very slowly through the membrane and for a considerable time during the dialysis a proportion was adsorbed into the membrane. The colour of the urine paled as the cobalt passed into and through the membrane. Boiling did not precipitate the metal. Lead acetate precipitated part of the cobalt but the total quantity could not be thrown out of solution by this means. Silver nitrate also precipitated part of the metal. In both cases, the colour became paler in correspondence with the amount of precipitation of metal. The cobalt containing urine did not form a sulphide on the addition of ammonium sulphide, but after boiling with nitric acid and neutralising with ammonia, the sulphide formed quite readily. 50 c.cm. of urine containing 17 mgrs. of cobalt was placed in two small cells connected with a syphon and a weak electric current was allowed to pass through the solution by means of platinum electrodes. After 24 hours, the syphon was clamped off in the middle, and the fluid in each half was emptied into the corresponding cell. The electrodes were washed with hydrochloric acid. It was found that the cobalt had not been ascertainably deposited on either electrode and that the quantity of metal was equally distributed between the two cells. A solution of cobalt chloride of the same strength was submitted to the same procedure. The cell corresponding to the negative pole contained over 10 mgrs. of cobalt while that corresponding to the positive electrode contained just under 7 mgrs.

The negative electrode was stained brown and 1·5 mgrs. of cobalt was dissolved off it, while the positive electrode was free from cobalt deposit. It thus appears that the cobalt in the urine is in a condition of complex combination and is not present as free cobalt ions. The behaviour of the urine is greatly in favour of the brown colour being due to a cobalt compound.

Pyrophoric reduced cobalt was suspended in serum and kept sealed up for many weeks, but no brown compound formed. When a cobalt salt such as the chloride is added to normal urine, no brown compound results and the cobalt can be combined with sulphur to form the sulphide, by adding ammonium sulphide without any previous heating with nitric acid. Several attempts were made to precipitate the compound or to crystallise it out of solution by the addition of various reagents, but without success.

VII. THERAPEUTIC EXPERIMENTS IN NICKEL CARBONYL POISONING.

It has been suggested that the administration of gases and other substances, which would dissociate nickel carbonyl and deposit nickel in an insoluble form, might lead to recovery after poisoning. The foregoing account however of the events occurring in nickel carbonyl poisoning makes it clear that attempts to convert the nickel compound into an insoluble one within the animal body will not alter the course of poisoning, as this has already taken place. It has been shown that even nickel sulphide is dissociated in the tissues and acts toxically. However it was considered necessary to conduct experiments in this direction, but the results coincide with the theoretical conclusions arrived at. Especial attention was paid to experiments aiming at the formation of the sesquioxide of nickel with bromine, as one of the assistants in Dr Mond's laboratories asserted that he always found relief in the inhalation of bromine after he had been exposed to nickel carbonyl vapour. No animals were saved by this treatment. The same may be said of chlorine vapour. The compounds used in these experiments included the acid phosphate of sodium, the triphosphate of sodium and the pyrophosphate of sodium, sulphuretted hydrogen and ammonium sulphide (poly-sulphides), the ferri and ferro-cyanide of potassium, the cacodylate of sodium and atoxyl, etc. In none of the experiments was a curative action discernible, and it must be assumed that either the more insoluble compound was not formed or that if it was formed it was again dissociated.

Better results attended a purely symptomatic treatment. The object aimed at was to keep the animals under the influence of narcotics and sedatives in order to slow down the circulation and respiration and thus to slow down the rate of absorption of the nickel. It was noticed that in some of the untreated animals, recovery had taken place when the animals remained quite quiet, while others which were apparently less severely affected died if they indulged in exercise during the early stages of poisoning. Morphine was found to be unsuitable. Veronal (diethyl malonyl urea) too did not lead to satisfactory results. A series of experiments with neuronal (bromine diethyl acetamine) showed that if given in doses just sufficient to keep the animals drowsy the fatal result could be averted in a considerable number, provided that only minimal lethal doses had been applied. When the dose of nickel was increased, the treatment failed to save any animals. Twenty-nine cats were exposed to 0.04 volume per cent. of nickel carbonyl vapour in air for 7.55 minutes. Sixteen were treated with neuronal and 13 served as controls. Of the treated cats 9 died and 7 recovered giving 43.7 per cent. of recoveries while of the controls 11 died and 2 recovered, giving 15.3 per cent. of recoveries. The drug proved to be quite harmless, which could not be said of veronal and morphine, and it was found that it was quite easy to regulate the dosage, so that the cats were only drowsy.

One is therefore justified, in the absence of a better form of treatment, to suggest that any cases of poisoning with nickel carbonyl should be treated by complete rest in bed, and small doses of neuronal (starting with 0.5 gram) repeated until somnolence begins to manifest itself.

Treatment only proved of value in cases when an amount of nickel carbonyl which was about a fatal dose had been inhaled, so that it is imperative to adopt preventive measures in places where nickel carbonyl is dealt with. That this is possible has been demonstrated by the fact that since 1904, no cases of poisoning have occurred at the Mond Nickel Works in Clydach, where nickel carbonyl is being produced in large quantities. At these works, the precautions taken to prevent escapes, and also the maintenance of a violent upward draught, effectually prevent an accumulation of poisonous vapour.

VIII. SUMMARY AND CONCLUSIONS.

Nickel carbonyl poisoning is a particular instance of nickel poisoning.

The lethal dose of nickel varies according to the method of application. When applied by subcutaneous injection, the physical condition of the compound influences the rate of absorption and therefore relatively large quantities may be required. In rabbits, the lethal dose is about $7\frac{1}{2}$ mgrs. per kilogram body weight under the most favourable conditions when applied subcutaneously. In cats it is about $12\frac{1}{2}$ mgrs. per kilogram body weight. When applied intraperitoneally, the absorbing surface is considerably larger and consequently the dose required to kill is smaller. In rabbits it is less than 7 mgrs. When applied in the form of nickel carbonyl vapour one meets with the most favourable conditions for rapid absorption and the dose is therefore still smaller. Rabbits die after the absorption of between 3 and 4 mgrs., while cats die after absorbing about $8\frac{1}{2}$ mgrs. per kilogram body weight.

In the lungs, nickel carbonyl is dissociated and a nickel compound, probably the hydrated sub-carbonate, is deposited on the respiratory surface.

The nickel is dissolved from the respiratory surface by the tissue fluids and is then taken up by the blood.

Some of the nickel finds its way directly through the lymphatic channels into the bronchial glands.

In the dissolved condition, the nickel enters into complex combination with some constituent of the body.

The nickel is carried by the blood to the tissues, but a selective absorption is exercised by the brain and adrenals. In the case of other forms of nickel poisoning, the lungs also exert this specific selection. The nickel only stays for a short time in these organs.

The specific pathological changes which are produced by nickel in these organs are primarily a degeneration of the endothelial cells of the capillary vessels. It is possible that some further primary action is exercised on the ganglion cells in the brain and on the parenchyma cells of the adrenals.

The haemorrhages follow as the result of the fatty degeneration of the vessel walls and secondary changes result from the effects of the haemorrhages.

The nickel is excreted by the kidneys and intestines.

The method of poisoning with iron carbonyl is similar to that of nickel poisoning, but the amount necessary to kill in the former case is larger.

Iron carbonyl poisoning like nickel carbonyl poisoning is merely a specific instance of metallic poisoning.

Iron acts in a similar manner to nickel on the walls of capillary vessels, but no evidence of selection by any special tissues was obtained.

Cobalt has a toxicological action which is identical to that of nickel. The lethal dose however is higher than that of nickel and lower than that of iron.

After the inhalation of a quantity of nickel or iron carbonyl which is greater than the minimum required to kill, no form of treatment was found to avert death.

It is with much pleasure that I again express my gratitude to Dr Ludwig Mond, F.R.S., for having rendered this investigation possible, by defraying all the expenses, and by giving me the benefit of his advice.

I further desire cordially to thank Dr C. J. Martin, F.R.S., and the other members of the Staff of the Lister Institute, who have at all times been ready and willing to assist me. The work has necessitated incursions into several branches of science, and has required the acquisition of a variety of methods. I have made free use of their kind collegiality and am glad to avail myself of this opportunity of recording my indebtedness.

The literature of the subject has been given in Part I, q.v.

DESCRIPTION OF PLATES.

PLATE VII.

Fig. 1. Section of lung of rabbit, poisoned by $\text{Ni}(\text{CO})_4$, showing intra-alveolar haemorrhage, oedema and fibrinous exudation; intra-bronchial exudation with proliferation of epithelial cells; cellular infiltration of the peribronchial tissue can also be seen. (Leitz Oc. 5, Obj. 3.)

Fig. 2. Same specimen under higher magnification. The oedema and haemorrhage are more clearly seen. Degeneration of the alveolar cells is present. (Leitz Oc. 3, Obj. $\frac{1}{2}$ oil immers.)

Fig. 3. Section of lung of rabbit poisoned with $\text{Fe}(\text{CO})_5$, showing enormous haemorrhagic infiltration. The alveolar structure has been partially broken up and the cells are degenerate. Some leucocytosis is present. (Leitz Oc. 3, Obj. $\frac{1}{2}$ oil immers.)

Fig. 4. Section of lung of guinea-pig poisoned by subcutaneous injection of CoCl_2 . Showing well-marked oedema and fibrinous exudation, inter- and intra-alveolar haemorrhage, with breaking up of the alveolar structure. (Leitz Oc. 1, Obj. $\frac{1}{2}$ oil immers.)

PLATE VIII.

Fig. 5. Section of supra-renal gland of rabbit poisoned with $\text{Ni}(\text{CO})_4$ vapour. Well-marked haemorrhagic infiltration and early degeneration of the nuclei of the parenchymatous cells. (Leitz Oc. 3, Obj. $\frac{1}{2}$ oil immers.)

Fig. 6. Section of brain (close to the optic thalamus) of cat, poisoned by $\text{Ni}(\text{CO})_4$ vapour. Fixed by Marchi's osmic acid fluid and stained with haematoxylin and eosin. The specimen shows a small vein in transverse section. The wall of the vessel shows well-marked fatty degeneration. The fat globules are situated partly in the sub-endothelial space and partly in the endothelial cells. The latter are swollen, misshapen and poor in chromatin. (Leitz Oc. 3, Obj. $\frac{1}{2}$ oil immers.)

Fig. 7. Section of medulla oblongata of rabbit poisoned with $\text{Ni}(\text{CO})_4$ vapour, showing a capillary blood vessel giving way. The degenerative changes in the endothelial cells of the capillary wall are advanced; those cells which are still recognizable are swollen, indistinct and contain little chromatin. There is some leucocytosis in the neighbourhood of the capillary. (Leitz Oc. 3, Obj. $\frac{1}{2}$ oil immers.)

Fig. 8. Section of brain (mid-cerebrum) of rabbit poisoned with $\text{Ni}(\text{CO})_4$ vapour, showing chromatolysis of Nissl's granules in the ganglion cells. Many of the processes are broken off and the cells have become misshapen and have lost chromatin. Leucocytosis is also present. The vessel seen has not yet ruptured. (Leitz Oc. 3, Obj. $\frac{1}{2}$ oil immers.)

PLATE IX.

For description see text, p. 566.

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**Apparatus used for Experiments
on Nickel Carbonyl.**