REFERENCES.

Breinl, A. (XI, 1907), "On the Morphology and Life History of *Spirochæta duttoni*," 'Ann. Trop. Med. and Parasitol.,' vol. 1, pp. 435—438, 1 pl.

Dutton, J. E., and Todd, J. L. (XI, 1907), "A Note on the Morphology of *Spirochæta duttoni*," 'Lancet,' 1907 (ii), pp. 1523—1525.

Fantham, H. B. (VI, 1907), "*Spirochæta* (*Trypanosoma*) *balbianii* (Certes), its Movements, Structure, and Affinities; and on the Occurrence of *Spirochæta anodontæ* (Keysselitz) in the British Mussel, *Anodonta cygnea*," 'Ann. Mag. Nat. Hist.,' ser. 7, vol. 19, pp. 493—501 (prelim. communic.).

Fantham, H. B. (I, 1908), "*Spirochæta* (*Trypanosoma*) *balbianii* (Certes) and *Spirochæta anodontæ* (Keysselitz): their Movements, Structure, and Affinities," 'Quart. Journ. Microsc. Sci.,' vol. 52, pp. 1—73, 3 pls.

Mackinnon, D. L. (1909), "Observations on the Division of Spirochætes," 'Parasitology,' vol. 2, pp. 267—280.

Nuttall, G. H. F. (VIII, 1908), "Spirochætosis in Man and Animals" (Harben Lecture II), 'Roy. Inst. Publ. Health Journ.,' pp. 449—464.

Swellengrebel, N. H. (VII, 1907), "Sur la Cytologie comparée des Spirochètes et des Spirilles," 'Ann. Inst. Pasteur,' vol. 21, pp. 448—465, 562—586, 2 pls.

The Origin and Destiny of Cholesterol in the Animal Organism. Part VI.—*The Excretion of Cholesterol by the Cat.*

By G. W. Ellis and J. A. Gardner, Lecturer in Physiological Chemistry, University of London.

(Communicated by Dr. A. D. Waller, F.R.S. Received August 14, 1909.)

(From the Physiological Laboratory, South Kensington, University of London.)

In an earlier paper* of this series the results of a number of estimations of the cholesterol content of the fæces of a dog fed on a variety of diets—animal and vegetable—were described. It was shown that the cholesterol found in the case of meat diets could be entirely accounted for by that present in the food, and from a general survey of the whole of the results, the opinion was expressed that the whole of the cholesterol of the bile is not excreted in the fæces, and must therefore have been either totally destroyed or reabsorbed in the gut along with the bile salts. In the case of a diet of raw brain, it was found that the cholesterol was not excreted as such, but entirely in the form of coprosterol. This was subsequently† found to be the case when cats were fed on either raw or cooked brain.

* 'Roy. Soc. Proc.,' B, vol. 80, 1908.

† 'Roy. Soc. Proc.,' B, vol. 81, 1909.

A few months after the appearance of our paper, Chosaburō Kusumoto* published a series of estimations of the cholesterol content of the fæces of dogs fed on horseflesh, and horseflesh with the addition of measured quantities of fat bacon or carbohydrates. His results showed that the cholesterol content of the food was considerably greater than that of the fæces. He also found that on meat diets variable quantities of coprosterol were always excreted with the cholesterol, and that the proportion of coprosterol increased with the fat in the food. This explains our observations on brain diets, the putrefactive changes which cause the formation of coprosterol being favoured by the presence of fats. The daily outputs of cholesterol observed by Kusumoto are somewhat greater than in the case of the dog we used, but his daily rations were much larger. He gives no data to indicate the purity of the specimens of cholesterol weighed. As dogs are omnivorous feeders, it seemed desirable to examine the fæces of more truly carnivorous beasts, and for this purpose the cat was selected. The fæces collected during each diet period were dried in the water oven, roughly powdered, or, if too greasy, ground with plaster of Paris, and extracted thoroughly for many days in a Soxhlet's apparatus with ether. The ethereal extracts were treated in the manner fully described in our paper† on "The Cholesterol Contents of Eggs and Chicks."

Experiments in which cats were fed on meat diet:—

I. A cat, which had previously been fed on raw brain diet, for the purpose of another experiment, for 14 days, during which it went down in weight from 2·8 to 2·3 kilogrammes, was fed for 14 days on a diet of lean cooked horseflesh. During this period it devoured 3125 grammes of the meat, and increased in weight steadily, its weights taken every third day being 2·3, 2·5, 2·5, 2·7, and 2·9 kilogrammes. The total dried fæces weighed 98 grammes, and yielded after the treatment described 2·554 grammes of unsaponifiable matter in the form of an oily mass. This was crystallised from alcohol repeatedly, but only 0·2052 gramme of pure crystalline matter was obtained. This melted at 93°—99° C. and had the characteristic crystalline form of coprosterol. The mother liquors were evaporated to dryness and treated in pyridine solution with excess of benzoyl chloride. On pouring into water the benzoate of coprosterol was thrown out of solution, and on washing with a small quantity of alcohol was sufficiently pure for weighing. The total weight of coprosterol obtained was 1·397 grammes, which corresponds to an

* "Über den Cholesterolgehalt der Hundfäces bei gewöhnlicher Ernährung und nach Fütterung von Cholesterin," 'Biochemische Zeitschrift,' vol. 14, 1908, pp. 411 and 416.

† 'Roy. Soc. Proc.,' B, vol. 81, 1909, p. 129.

output of 0·098 gramme per day. If we take Dormeyer's* figure, 0·23, as the percentage of cholesterol in dry muscle, and assume that the cooked meat contained 62 per cent. of moisture,† the animal should have received in its food some 2·7 grammes of cholesterol. A considerable quantity of cholesterol, therefore, must have been absorbed by the animal, which was putting on flesh during the whole experiment.

II. A cat, which had previously been fed on a diet of cooked brain for 14 days, during which it lost weight from 2·9 to 2·5 kilogrammes, was fed for 14 days on lean cooked horseflesh. During this period it devoured 2940 grammes of the meat, and its weights taken every other day were 2·5, 2·5, 2·7, 2·8, 2·9, and 2·8 kilogrammes. The total weight of dried fæces was 92 grammes, which gave 3·02 grammes of unsaponifiable matter. On repeated crystallisation from alcohol, 0·588 gramme of pure coprosterol, melting at 93°—98° C., was obtained. The mother liquor on benzoylation yielded a further quantity of coprosterol benzoate. The total coprosterol thus obtained weighed 1·077 grammes, corresponding to an output of 0·077 gramme per day. Calculating as before, the animal received in its food 2·56 grammes of cholesterol.

III. In this experiment a healthy cat was fed for 14 days on 1550 grammes of lean cooked meat—beef and mutton. Its weight remained practically constant during the experiment. 44 grammes of dry fæces were obtained, which yielded 1·331 grammes unsaponifiable matter. On treatment this gave 0·27 gramme of white crystalline matter, melting at 129°—132°, which appeared to be a mixture of cholesterol and coprosterol. A further quantity as benzoate was isolated from the mother liquors. Total weight of cholesterol and coprosterol, 0·5486, corresponding to an output of 0·032 gramme per day. Calculating as before, the animal received in its food 1·35 grammes of cholesterol.

IV. As the experiments described are possibly open to the criticism that the sameness of the diet over a long period may have affected the metabolism, in this experiment we took four cats and fed them for *seven* days on a diet of raw lean bullock's heart. The animals appreciated this food and took it greedily. They consumed in seven days 5166 grammes, the daily ration of each animal being of the same weight. On the eighth day each animal had a meal of cooked wheat germ, which had previously been freed from fat and phytosterol by extraction with ether, in order to sweep out the gut. The fæces were very oily in character and difficult to dry at

* 'Pflüg. Archiv,' 1896, vol. 65, p. 99.

† A sample of this cooked horseflesh was dried at 100° C. and found to contain 62 per cent. of moisture.

80° C. The weight of partially dry stuff was 362 grammes. The weights of the animals were taken at the beginning of the experiment and periodically afterwards with the following result:—

	At beginning.		On third day.		On last day.	
	lbs.	ozs.	lbs.	ozs.	lbs.	ozs.
Cat I.....	8	2	7	14	7	12
„ II.....	7	2	6	14	7	2
„ III.....	6	2	6	1	5	5
„ IV.....	5	4	5	4	5	6

The fæces were extracted in a Soxhlet's apparatus with ether for 19 days and yielded 4·3985 grammes of unsaponifiable matter. This was a dark stiff vaseline-like substance. The unsaponifiable matter was repeatedly crystallised from alcohol, but it proved exceedingly difficult to purify. Eventually 0·05 gramme pure coprosterol, melting 99°—102°, was obtained. The impure crystalline crops and all the mother liquors, after evaporating to dryness, were separately treated in pyridine solution with excess of benzoyl chloride. On pouring into water the crude benzoates which separated were treated in a similar manner to that described in a former paper.* 1·7272 grammes of benzoate was obtained. This melted at 125°—128° without showing any play of colours. It appeared likely that we were dealing with a mixture of cholesterol and coprosterol benzoates, and the substance was, therefore, fractionally crystallised from ethyl acetate. The first crop of crystals on heating began to soften at 130° C. and melted to a turbid liquid at 141° C., which became clear at 165° C. On cooling, the play of colours characteristic of cholesterol benzoate was shown in a well-marked manner. A microscopic examination showed that it consisted of the characteristic plates of cholesterol benzoate with a comparatively small quantity of coprosterol benzoate. A later crop, which a microscopic examination showed to consist mainly of coprosterol benzoate with only a very few crystals of cholesterol benzoate, melted at 117°—119° to a clear liquid. Pure coprosterol benzoate melts at 120°—121° C. Evidently, therefore, a mixture of cholesterol and coprosterol was excreted, the total weight being 1·4004 grammes, corresponding to an output per day per cat of 0·05 gramme.

Heart muscle contains between 0·066 and 0·071 per cent. of cholesterol,† so that the animals received in their food about 3·5 grammes of cholesterol.

* "Origin and Destiny of Cholesterol in the Animal Organism," Part IV, 'Roy. Soc. Proc.,' B, vol. 81, p. 129.

† "Cholesterol Content of Heart Muscle," 'Journ. Physiol.,' vol. 38, 1908, 'Proc.,' p. 1.

The results of these experiments are summarised in the following table:—

Experiment.	Total cholesterol taken in food.	Duration of diet period.	Total weight of unsaponifiable matter.	Total weight of cholesterol and coprosterol passed in fæces.	Deficit.	Output per cat per day.
	grammes.		grammes.	grammes.	grammes.	
I	2·7	14	2·554	1·397	1·303	0·098
II	2·56	14	3·02	1·077	1·483	0·077
III	1·35	14	1·331	0·5486	0·8	0·032
IV	3·5	28	4·3985	1·4004	2·099	0·05

It is clear from these results that cats behave similarly to dogs when fed on meat diets, but the tendency for the change of cholesterol into coprosterol appears to be greater in the case of cats.

The total cholesterol of the food should of course be increased by that poured into the gut in the bile during digestion. No data are, however, available for forming any estimate of these quantities.

In the hope of ascertaining whether the whole or any of the cholesterol of the bile was excreted in the fæces in the case of artifical diets as free as possible from cholesterol or phytosterol, and if so, whether under such conditions any reduction to coprosterol took place, the experiments detailed below were undertaken. We had some difficulty in finding suitable food, as cats are dainty animals and will not eat freely of substances that are in the least distasteful to them, and we thought that any attempt to starve an animal into eating any particular food would be likely to vitiate the results. Further, it was necessary that the diet should contain all the constituents required to keep the animal in good condition.

Ultimately the following diets were selected:—

(1) 90 grammes of white bread mixed with the white of one egg were moistened with a dilute solution of Liebig's extract of meat and lightly fried. About 4 grammes of cream were then added.

(2) About 200 grammes of germ of wheat, which had previously been thoroughly extracted with ether, were mixed with a little Liebig's extract dissolved in hot water to a stiff paste. This was incorporated with about 30 grammes of suet, and the paste baked for two or three hours in a dish in a hot oven. The suet used was purified as far as possible from cholesterol by dissolving in ether and precipitating with alcohol several times. An analysis showed that this purified fat still contained 0·118 per cent. of cholesterol either free or in the form of esters.

The animals experimented on partook of these foods readily and appeared to thrive on them.

V. A cat weighing 3·5 kilogrammes was fed for 17 days on the above-mentioned bread and egg diet, and the fæces were collected during the last 15 days. During the period in which fæces were collected, the animal ate 1390 grammes of bread, the whites of 18 eggs, and about 65 grammes of cream. The weight of the cat remained quite constant until the 10th day of the experiment, after which it gradually decreased to 3·2 kilogrammes; 300 grammes of dry fæces were collected, which after extraction yielded 1·735 grammes of unsaponifiable matter as a dark oil. On recrystallisation from alcohol three times, 0·6075 gramme of white crystalline matter, melting at 125°—138° C. was obtained. From the residues 0·2604 gramme of benzoate was prepared. A microscopic examination of the crystalline matter showed that it was a mixture of cholesterol with probably some phytosterol-like substance from the bread. Reckoning the whole as cholesterol, the total weight obtained was 0·8126 gramme, corresponding to an output of 0·05 gramme per day.

VI. This cat was fed for 17 days on the bread-egg-cream diet, with the addition during part of the time of 2 grammes of cholesterol, given in 0·25-gramme portions. Altogether the animal ate 1710 grammes of bread, the white of 17 eggs, and about 60 grammes of cream. It liked the food, and during the experiment increased in weight from 3·2 to 3·3 kilogrammes. The total weight of dry fæces was 488 grammes and yielded 2·48 grammes of unsaponifiable matter of a crystalline nature. After twice crystallising from alcohol, 1·935 grammes of white crystalline matter were obtained. This was again recrystallised from alcohol, and the main crop consisted of almost pure cholesterol, melting at 143°—144° C. The final mother liquors yielded a minute amount of matter, crystallising in star-shaped aggregates of needles, not unlike coprosterol in appearance. The residues, on benzoylation in pyridine, yielded 0·1723 gramme of benzoates. Assuming that the whole crystalline matter consisted of cholesterol, the total amount was 2·07 grammes, a quantity only a little greater than the weight of pure cholesterol given to the animal.

VII. Four cats were fed for 10 days on the above-mentioned diet of extracted germ of wheat and purified fat, the fæces being collected during the last nine days. The animals took the food readily and ate during the period 1980 grammes of wheat germ and 308 grammes of fat, the total weight of which, when cooked as described, was 3916 grammes. The weights of the cats during the experiments were as follows.

490 grammes of dry fæces were collected and yielded on extraction 3·3246 grammes of unsaponifiable matter of an oily semi-solid consistency.

After several crystallisations from alcohol, 1·1495 grammes of white

	First day.	Third day.	Sixth day.	Ninth day.
	lbs. ozs.	lbs. ozs.	lbs. ozs.	lbs. ozs.
Cat I.....	7 14	7 14	8 0	8 2
„ II.....	7 6	7 6	7 4	7 2
„ III.....	6 14	6 10	6 4	6 2
„ IV.....	5 14	5 12	5 6	5 4

crystalline matter were obtained, which melted at 135°—137° C. This consisted mainly of cholesterol, for a portion, after recrystallisation again from alcohol, melted at 142° C., and another portion, on treatment in ether acetic acid solution with bromine, according to Windaus' method, gave cholesterol dibromide, melting at 120°—122° C., in fair yield. The mother liquors, after recrystallisation from alcohol, yielded a small amount of matter, which under the microscope had the appearance of a mixture of cholesterol and phytosterol. The residues, after separating the above-mentioned 1·1495 grammes of cholesterol, were benzoylated in pyridine solution and 0·5049 gramme of fairly clean benzoate was obtained. This melted, after recrystallisation from ethyl acetate at 146°—147° C., to a turbid liquid, which cleared at 170° and on cooling gave a brilliant display of colours. Reckoning the whole of the crystalline matter as cholesterol, 1·5472 grammes were obtained, corresponding to a daily output of about 0·04 gramme; or, if we subtract the quantity of cholesterol contained in the fat given with the wheat germ, which amounted in all to 0·36 gramme, the daily output, independent of food, was 0·033 gramme. No trace of coprosterol was discovered.

VIII. A cat, weighing 1·7 kilogrammes, was fed on a diet prepared similarly to the last, but without any fat, for 17 days. It consumed altogether 630 grammes of extracted germ of wheat, and produced 93 grammes of dried fæces. The weight of the unsaponifiable matter was 0·6930 gramme and fairly crystalline. From this 0·5495 gramme of cholesterol was obtained, corresponding to an output per day of about 0·03 gramme.

IX. A cat was fed as in Experiment VIII for eight days, but during the first five days it received, mixed with its food, small quantities of pure phytosterol. It consumed altogether 250 grammes of the extracted germ and 1·41 grammes of phytosterol. The weight of dry fæces was 68 grammes and this yielded 1·7415 grammes of unsaponifiable matter as a greasy crystalline solid. On crystallisation from alcohol, 1·3545 grammes of white crystalline matter, which appeared to consist of almost pure phytosterol. An attempt was made to separate any cholesterol from this by conversion into the dibromides by Windaus' method, but without success. A small quantity of dibromide separated out on standing after the addition of the acetic acid

solution of bromine to the solution of the substance in ether; this was filtered off and reduced in glacial acetic acid solution with zinc dust. The product, which should have been cholesterol, had it been present in any quantity, on heating began to soften at 137° and was not completely melted until 142° C. An examination of the crystals under the microscope showed that they contained phytosterol. The soluble dibromide treated in a similar way gave a substance melting at 138°—140° C. A microscopic examination showed that this was largely phytosterol.

The residues, after the separation of the 1·3545 grammes of phytosterol, were treated in pyridine solution with benzoyl chloride; 0·1196 gramme of benzoate was obtained. This, after recrystallisation from alcohol, melted at 145°—146° C. to a *clear* liquid and on cooling showed colours, though not very brilliantly. On carefully examining the crystals under the microscope, they were found to consist of phytosterol benzoate and none of the typical square plates of cholesterol benzoate could be seen. The conversion of phytosterol into the benzoate by the pyridine method is by no means quantitative, so that we do not know whether all was recovered from the residues. Altogether 1·4487 grammes of phytosterol were recovered, including, of course, cholesterol if present. All the phytosterol given in the food was, therefore, excreted unchanged, but whether accompanied by cholesterol we cannot say. There could not, however, have been much.

X. Immediately after the conclusion of the last experiment the diet was continued, but with the substitution of cholesterol for phytosterol. The experiment lasted 12 days, cholesterol being given with the food on the first eight days only. During the period the animal ate 620 grammes of germ and 2 grammes of cholesterol; 138 grammes of dried fæces were collected and the yield of unsaponifiable matter was 2·1775 grammes in the form of a brown solid. After recrystallising twice from alcohol, 1·5455 grammes of cholesterol, melting at 145°—147° C., were obtained. This figure is rather low owing to an accident, but the amount lost was under one-tenth of a gramme. The residues on benzoylation yielded 0·4386 gramme of cholesterol benzoate. The total cholesterol obtained was, therefore, 1·89 grammes, so that the total amount excreted could not have been greater than the weight of the cholesterol administered.

Discussion of the Results.

The conversion of cholesterol into coprosterol in the gut of the cat appears to take place only in the case of meat diets, and then the change is not necessarily complete. The two cats in Experiments I and II which yielded only coprosterol had been previously fed for some time on sheep's brain. The

others, which had previously been fed in an ordinary way and led the ordinary domestic life, yielded a mixture of cholesterol and coprosterol. The animals fed on the artificial and vegetable diets gave no coprosterol. This recalls the experiences of Müller,* who found that in man a prolonged milk diet resulted in the excretion of cholesterol and not coprosterol. In all our experiments on meat diets the total cholesterol and coprosterol excreted was considerably less than that taken in with the food. Without considering the cholesterol poured into the gut with the bile, the percentage loss in Experiments I and II together was 53 per cent.; in Experiment III, 40 per cent.; and in Experiment IV, between 59 and 60 per cent., an average loss of 0·08 gramme per day. Two alternative explanations of these results suggest themselves.

(1) The hypothesis put forward in an earlier paper† that cholesterol is a substance which is strictly conserved in the animal economy: that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is reabsorbed in the intestine along with the bile salts, and finds its way into the blood stream to be used in cell-anabolism; and further, that any waste of cholesterol might be made up from that taken in with the food. This latter process would of course be limited in man and carnivorous animals by the change of cholesterol into coprosterol, and in herbivorous animals by the fact that their normal food does not contain cholesterol, but isomeric substances such as phytosterol, which would have to be converted into cholesterol before utilisation. Further evidence in support of this hypothesis in the case of herbivorous animals was brought forward by Miss Fraser and one of us in a paper‡ on the "Inhibitory Action of the Sera of Rabbits fed on Diets containing varying Amounts of Cholesterol on the Hæmolysis of Blood by Saponin."

(2) The change of cholesterol into coprosterol is generally supposed to be one of simple reduction brought about in the intestine by the bacteria of putrefaction. We have, however, no experimental evidence that coprosterol is a simple reduction product of cholesterol, as it is quite different from any of the bihydrocholesterol derivatives hitherto produced in the laboratory; and further, attempts to bring about the change *in vitro* by means of bacteria have so far been unsuccessful. Whatever the exact nature of the change

* "Reduction of Cholesterol to Coprosterol in the Human Intestine," 'Zeit. physiol. Chem.,' 1900, vol. 29, pp. 129—135.

† "Origin and Destiny of Cholesterol," Part III, 'Roy. Soc. Proc.,' B, vol. 81, 1909, p. 109.

‡ "Origin and Destiny of Cholesterol," Part V, 'Roy. Soc. Proc.,' B, vol. 81, 1909, pp. 230—247.

may be, however, it may be accompanied either by a total destruction of a portion of the cholesterol, which in view of the great chemical stability of the molecule of this substance is unlikely, or a change of a portion into some non-crystalline oily product.

We do not think, however, that a comparison of the total weights of the unsaponifiable matter of the fæces given in Table I with the weights of cholesterol in the food bears out the second explanation, more especially when we remember that the latter weights should be increased by the quantities of cholesterol poured into the gut with the bile during digestion. The weights of unsaponifiable matter are, moreover, generally higher than the truth, as they are often rather difficult to dry, without drastic means, and often contain traces of soap.

If the first-mentioned explanation were strictly true, we should not have expected to find any cholesterol in the fæces of the cats fed on the artificial cholesterol-free diets—the fæces should have been cholesterol-free, just as are those of herbivorous animals. Small quantities of cholesterol were, however, found. In Experiment V, on bread, egg, and cream diet, the cat excreted 0·05 gramme per day, a minute fraction of which, however, may have been due to cream ; and further, in this weight is included the phytosterol of the bread. In the case of the cats on extracted germ of wheat, in Experiments VII and VIII, the quantities excreted were 0·033 and 0·03 gramme per day respectively. These values may also have contained traces of phytosterol left in the germ after extraction. Whether these quantities are large enough to represent the whole of the cholesterol of the bile daily poured into the intestine, no data are available to determine. If, however, we adopt the data given for dogs, the values are undoubtedly too low. Further, the quantities of fæces produced per day on the vegetable diets were very much larger for a given weight of food than in the case of meat diets, and possibly this may have caused some of the cholesterol to escape absorption.

In the case of Experiments VI, IX, and X, however, in which known quantities of cholesterol or phytosterol were added to the daily rations of the artificial foods, no excess of cholesterol above that administered was recovered from the fæces.

From the point of view of deciding whether in the case of carnivorous animals the cholesterol of the bile is normally reabsorbed along with the bile salts in the intestine, these results are inconclusive. Experiments are, however, in progress to compare the effect on the blood of the addition of cholesterol to artifical diets such as those used in the experiments detailed in this paper. The results of these experiments we expect to give more

definite information on this point, and we hope to make the subject of a communication in the near future.

The expenses in connection with this work were defrayed by means of a grant made by the Government Grant Committee of the Royal Society, for which we take the opportunity of expressing our thanks.

On the Supposed Presence of Carbon Monoxide in Normal Blood and in the Blood of Animals anæsthetised with Chloroform.

By G. A. Buckmaster and J. A. Gardner.

(Communicated by A. D. Waller, M.D., F.R.S. Received August 12, 1909.)

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

While engaged in the study of the gases of the blood during the various stages of anæsthesia by chloroform, we found after absorption of the carbon dioxide and oxygen extracted by the blood pump an amount of residual gas far in excess of any amount that could be regarded as nitrogen remainder plus leak of apparatus.* We have found as the result of many experiments, carried out to determine this particular point, that practically all the chloroform present in the blood of anæsthetised animals comes off with the gases of the blood when these are extracted at 40° C., so that the excessive residual gas is in large part chloroform vapour, or its decomposition products. The exact method of procedure of analysis of these gases and the effects of the presence of this chloroform on the methods of analysis will form the subject of a forthcoming paper, but we quote the following experiments to show what percentages of chloroform may be present:

Cat, weight 3 kilos.; chloroformed for one hour with an air-chloroform mixture 2—3 per cent., 54 c.c. of dark blood withdrawn from carotid artery.

The gases extracted with the pump at 40° C. were mixed with excess of pure moist oxygen and passed through red-hot spiral platinum tubes. The products of combustion were collected in ammonia. This was exactly neutralised with nitric acid and titrated with silver nitrate (1 c.c. = 0·001 Cl); 17 c.c. of silver nitrate were required = 0·01986 gramme $CHCl_3$ = 3·7 c.c. of chloroform vapour at 0° and 760 mm.

* The blood pump employed was the Tœpler as modified by Barcroft ('Journal of Physiology,' vol. 25, p. 265), with certain modifications for this particular work. These will be described in our paper on the blood gases in chloroform anæsthesia.