"The Influence of Removal of the Large Intestine and increasing Quantities of Fat in the Diet on general Metabolism in Dogs." By VAUGHAN HARLEY, M.D., Professor of Pathological Chemistry, University College, London. Communicated by Prof. VICTOR HORSLEY, F.R.S. Received July 25, 1898.

(Abstract, published during the Vacation.)

In this research it was intended, by comparing the results obtained in dogs on a given diet with the same animals after the removal of the large intestine, to study more carefully the functions and any influence the absence of the large intestine might have on general metabolism.

In one dog a little more than the middle third of the large intestine was removed, while in the other two dogs the total length of the large intestine, together with the cæcum, was extirpated. The dogs were fed after recovering from the operation on meat and biscuit, to which varying quantities of fat were added. The meat employed was preserved by sterilising minced meat in separate weighed out portions sufficient for each day.

In all the experiments the nitrogen and fat in the diet were analysed, and in two dogs quantitative analyses were made of the carbohydrates in the diet and fæces. It was found on the above diet that no carbohydrates were obtainable in the fæces either in normal dogs or in those in which the large intestine had been removed, so that it can be concluded that the large intestine has no action on the carbohydrate absorption, and in subsequent experiments it was not investigated.

The first step was to investigate the effect of an increasing quantity of fat on a staple diet in normal animals, so as to compare that with the results obtained after the removal of the large intestine.

In dog 1 (Table III) it is seen on the same carbohydrate and proteid diet when the quantity of fat is increased from 12·04 grams to 32·04 grams, the average quantity of urine fell from 118 c.c. to 89 c.c., and this decrease in quantity was accompanied by a slight increase in the specific gravity of 1058 to 1060. In consequence of the proteid sparing action of the fat the quantity of nitrogen eliminated fell from 4·457 grams to 3·575 grams. On increasing the diet still further to 62·04 grams, the quantity of urine was only 70 c.c., the specific gravity remaining 1060, the nitrogen being slightly decreased in quantity to 3·362 grams.

As far as the fæces are concerned, their daily quantity increased from 18·61 grams to 20·42 grams and 22·70 grams. Together with the increased quantity of the fæces the nitrogen daily eliminated also increased in quantity from 0·351 gram to 0·412 gram and 0·469 gram. As one would naturally expect, with the increased quantity of fat in the diet, the fat in the fæces increased from 0·733 gram to 0·971 gram

Table III.—The Effect of an Increasing Quantity of Fat in the Diet on Normal Metabolism, giving the Averages of Periods of Four Days Duration.

| No. | Weight. | Duration of observation. | Diet. | | Urine. | | | Fæces. | | | Absorbed. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | N. | Fat. | Quantity. | Sp. gr. | N. | Quantity. | N. | Fat. | N. | Fat. |
| | kilos. | days. | grams. | grams. | c.c. | | grams. | grams. | grams. | grams. | per cent. | per cent. |
| 1*a* | 4·59 | 4 | 4·82 | 12·04 | 118 | 1058 | 4·457 | 18·61 | 0·351 | 0·733 | 92·71 | 93·91 |
| *b* | 4·59 | 4 | 4·82 | 32·04 | 89 | 1060 | 3·575 | 20·42 | 0·412 | 0·971 | 91·45 | 96·97 |
| *c* | 4·63 | 4 | 4·82 | 62·04 | 70 | 1060 | 3·362 | 22·70 | 0·469 | 1·264 | 90·26 | 97·96 |
| 2*a* | 6·41 | 4 | 8·00 | 15·20 | 119 | 1054 | 6·127 | 31·67 | 0·696 | 0·776 | 91·29 | 94·68 |
| *b* | 6·41 | 4 | 8·00 | 15·20 | 108 | 1059 | 5·815 | 33·62 | 0·799 | 0·898 | 90·01 | 94·09 |
| *c* | 6·59 | 4 | 8·00 | 65·19 | 74 | 1061 | 3·858 | 36·26 | 0·901 | 2·249 | 88·73 | 96·55 |

and 1·264 gram. In consequence of the increase of the nitrogen in the fæces, the apparent absorption of nitrogen per diem fell from 92·71 per cent. to 91·45 per cent. and 90·26 per cent., while, on the other hand, in spite of the increased quantity of fat in the fæces, the absorption of fat rose from 93·91 per cent. to 96·97 per cent. and 97·96 per cent. by increasing the fat in the diet.

In dog 2 during the three periods examined, the first two were on the same quantity of fat, and the results found correspond with those found in dog 1.

The next observation (Table V) was made on a dog in which a little more than one-half of the large intestine was removed. It is seen that on increasing the fat from 11·73 to 36·73 grams the quantity of urine fell from 172 c.c. to 169 c.c., the specific gravity falling from 1035 to 1031. The nitrogen also fell from 5·596 grams to 4·991 grams. On still further increasing the fat to 51·73 grams, the average quantity of urine fell to 112 c.c. with a specific gravity of 1048, the quantity of nitrogen being 4·680 grams when four days were analysed. As far as the quantity of fæces is concerned, during two of the periods the dog did not pass its fæces daily, so that the average can only be made approximately.

The increase in the quantity of fat is seen in this case to cause no increase in the quantity of fæces, although the total quantity of fæces are more than the previous normal dogs would lead one to suppose ought to occur on that diet. The total nitrogen in the fæces also is increased in quantity, although no increase occurs with the addition of the fat. The fat analysis in the three periods remained practically the same; in consequence of this, the absorption is not altered by increasing the fat. The total absorption of proteid varies from 86·91 per cent. to 89·85 per cent., that is to say less than the normal dogs, while the fat absorption rose from 86 per cent. to 97 per cent., practically the same as is found when the large intestine is intact.

In the next two dogs the entire large intestine was removed, and the result obtained is seen in Table VIII.

In dog 4, four periods were analysed, each having four days duration. During periods *a* and *b* the quantity of fat was 9·71 grams, while in periods *c* and *d* the quantity was 29·71 grams.

It is seen that the quantity of urine during the periods *a* and *b* varied very considerably, and the same occurred with the increased quantity of fat. The cause of this variance it would be impossible to explain; the tendency, however, is for the quantity to slightly decrease with the increased quantity of fat, and specific gravity to do the same.

At the same time the decrease in quantity on increasing the fat is not nearly so marked as in the normal dogs. The increase of fat in the diet, however, brings out the same proteid sparing action as the

Table V.—The effect on increasing the quantity of Fat in the Diet on General Metabolism is seen after partial removal of Large Intestine. Averages of Three Periods of Four to Five Days Duration. Figures in ( ) not complete averages owing to an analysis in each case being lost.

| No. | Weight. | Duration of observation. | Diet. | | Urine. | | | Fæces. | | | Absorbed. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | N. | Fat. | Quantity. | Sp. gr. | N. | Quantity. | N. | Fat. | N. | Fat. |
| | kilos. | days. | grams. | grams. | c.c. | | grams. | grams. | grams. | grams. | p. c. | p. c. |
| 3*a* | 6·18 | 5 | 6·05 | 11·73 | 172 | 1036 | 5·596 | 39·95 | 0·792 | (1·217) | 86·91 | (86) |
| *b* | 6·23 | 4 | 6·05 | 36·73 | 169 | 1031 | 4·991 | 36·39 | 0·614 | 1·437 | 89·85 | 96·09 |
| *c* | 6·75 | 5 | 6·05 | 51·73 | 112 | 1048 | (4·689) | 38·31 | 0·624 | (1·492) | 89 69 | (97) |

Table VIII.—The effect of an increasing quantity of Fat in the Diet on General Metabolism in two Dogs in which the entire large intestine had been previously removed. Averages of Four Periods of Four Days, and One of Five and One of Two Days duration. Figures in ( ) not complete averages.

| No. | Weight. | Duration of observation. | Diet. | | Urine. | | | Fæces. | | | Absorbed. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | N. | Fat. | Quantity. | Sp. gr. | N. | Quantity. | N. | Fat. | N. | Fat. |
| | kilos. | days. | grams. | grams. | c.c. | | grams. | grams. | grams. | grams. | p. c. | p. c. |
| 4*a* | 4·05 | 4 | 6·80 | 9·71 | 191 | 1025 | 4·445 | 75·55 | 1·064 | 0·777 | 84·36 | 92·00 |
| *b* | 4·08 | 4 | 6·80 | 9·71 | 341 | 1014 | 4·374 | 74·71 | 1·081 | 0·605 | 84·11 | 93·77 |
| *c* | 4·17 | 4 | 6·80 | 29·71 | 207 | 1019 | 3·243 | 83·78 | 1·088 | 0·769 | 84·01 | 97·41 |
| *d* | 4·29 | 4 | 6·80 | 29·71 | 198 | 1023 | 2·965 | 93·49 | 1·095 | 0·873 | 84·14 | 97·06 |
| 5*a* | 6·30 | 5 | 6·26 | 11·55 | 93 | 1044 | 4·376 | 74·16 | 1·031 | (0·553) | 83·54 | (95) |
| *b* | 6·58 | 2 | 6·26 | 41·55 | 91 | 1028 | 2·415 | 59·38 | 0·696 | 0·819 | 90·33 | 98·03 |

quantity of nitrogen in the urine falls from 4·445 grams and 4·374 grams to 3·243 grams and 2·965 grams.

The quantity of fæces during the different periods compared very much better than the quantity of urine, and on a small fat diet the quantity was 75·55 grams and 74·71 grams, that is to say, more than double the quantity on the same diet in normal dogs. On increasing the fat in the diet the quantity rose to 83·78 grams and 83·49 grams, so that we have here the same as in the normal dogs an increase in the quantity of fæces caused by increasing the quantity of fat in the diet. At the same time the total quantity of fæces is very much in excess of that on the same diet in normal dogs.

The nitrogen in the fæces during the periods *a* and *b* was 1·064 grams and 1·081 grams, nearly three times the amount obtained in normal dogs, while on increasing the fat it only increased slightly to 1·088 grams and 1·095 grams. The fat in the fæces during periods *a* and *b* was 0·777 gram and 0·605 gram, and on increasing the fat in the diet it rose to 0·769 gram and 0·873 gram, so that the total quantity of fat obtained in the fæces in dogs in which the large intestine had been removed was almost the same as in the normal dog, and the increase of fat in the diet caused an increase in the quantity of fæces.

Now, turning to the absorption as indicated by the quantity found in the fæces, we see while the normal dogs absorbed over 90 per cent. of the nitrogen given, in the absence of the large intestine only 84 per cent. was absorbed, and that the increase in the quantity of fat given caused very little decrease in the absorption of nitrogen per cent. The absorption of fat in the normal dogs varied from 93 per cent. to 97 per cent., while in these dogs we see that it varies from 92 per cent. to 97 per cent., so that as far as the absorption of fat is concerned the large intestine plays no part.

In dog 5, in period *b*, when 41·55 grams of fat were given, the animal refused to take its food, so that only two days' analysis were able to be given; but the results obtained in dog 5 correspond with those in dog 4, and it was found in other dogs that it was impossible to increase the quantity of fat in the diet in the absence of the large intestine, as the animals invariably went off their feed.

The above results showed that the removal of the large intestine has a great influence on the quantity of fæces, it will be as well now to discuss the change in the quantity of water eliminated in the fæces and its percentage composition.

On comparing the averages of the above dogs we see (Table XIV) that in the normal dog the quantity of fæces varies from 18·61 grams to 36·26 grams, while when the large intestine is partially removed the quantity is slightly increased, although the small quantity found may be partly explained by constipation. On removal of the whole of the

Table XIV.—The Influence of an increasing Quantity of Fat in the Diet on the Quantity of Water in the Fæces.

| No. | Duration of observation. | Diet. | | Fæces. | | |
|---|---|---|---|---|---|---|
| | | N. | Fat. | Quantity. | Water. | |
| | days. | grams. | grams. | grams. | Total grams. | per cent. |
| | Average of Two normal Dogs. | | | | | |
| 1*a* | 4 | 4·82 | 12·04 | 18·61 | 12·79 | 70·78 |
| *b* | 4 | 4·82 | 32·04 | 20·42 | 13·79 | 67·95 |
| *c* | 4 | 4·82 | 62·04 | 22·70 | 14·32 | 67·87 |
| 2*a* | 4 | 8·00 | 15·20 | 31·67 | 19·90 | 63·26 |
| *b* | 4 | 8·00 | 15·20 | 33·62 | 22·03 | 64·60 |
| *c* | 4 | 8·00 | 65·19 | 36·26 | 23·67 | 60·52 |
| | Average of partial Removal of Large Intestine. | | | | | |
| 3*a* | 5 | 6·05 | 11·73 | 39·95 | 25·44 | 50·96* |
| *b* | 4 | 6·05 | 36·73 | 36·39 | 26·44 | 54·59* |
| *c* | 5 | 6·05 | 51·73 | 58·31 | 28·30 | 72·46 |
| | Average of total Removal of Large Intestine. | | | | | |
| 4*a* | 4 | 6·80 | 9·71 | 75·55 | 58·11 | 76·59 |
| *b* | 4 | 6·80 | 9·71 | 74·71 | 56·87 | 76·26 |
| *c* | 4 | 6·80 | 29·71 | 83·78 | 66·56 | 79·45 |
| *d* | 4 | 6·80 | 29·71 | 83·49 | 66·09 | 79·13 |
| 5*a* | 5 | 6·26 | 11·55 | 74·16 | 58·57 | 79·09 |
| *b* | 2 | 6·26 | 41·55 | 59·38 | 49·49 | 83·62 |

* One day passed no fæces.

large intestine, on the other hand, the quantity of fæces varies from 75 grams to 83 grams; that is to say, was very markedly increased.

The quantity of water in the normal fæces per diem is increased on increasing the quantity of fat in the food.

In dog 1 it rose from 12·79 grams to 14·32 grams. After removing the large intestine there is also an increase in the quantity of water on increasing the quantity of fat in the diet, for it rose from 58·11 grams and 56·87 grams to 66·56 grams and 66·09 grams by increasing the fat in the diet. Also the percentage quantity eliminated with the fæces is enormously increased by removal of the large intestine. Even the partial removal of the large intestine in dog 3 shows a very marked increase in the quantity of water per diem. The percentage of water in the fæces in the normal dogs slightly falls with the increased quantity of fat in the diet, whilst instead of falling the percentage increases after the removal of the large intestine. In the normal dog 70·78 per

Table XX.—The Effect of the Removal of the Large Intestine on the breaking up of Fat in the Alimentary Canal. Composition of Fat of Fæces.

| No. | Duration of observation. | Total ether extract. | | Neutral fat. | | Free fat acids. | | Fat acids as soaps. | | Cholesterin. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total. | Per cent. | Total. | Per cent. | Total. | Per cent. | Total. | Per cent. | Total. | Per cent. |
| | days. | | | | | | | | | | |
| | | | | | Average of Two normal Dogs. | | | | | | |
| 1 | 4 | 1·264 | 100 | 0·139 | 10·03 | 0·902 | 65·47 | 0·233 | 14·22 | 0·154 | 11·80 |
| 2 | 3 | 2·325 | 100 | 0·314 | 13·63 | 1·359 | 59·45 | 0·591 | 24·12 | 0·061 | 2·80 |
| | | | | | Average of partial Removal of Large Intestine. | | | | | | |
| 3 | 3 | 0·972 | 100 | 0·145 | 13·16 | 0·514 | 54·74 | 0·178 | 15·91 | 0·145 | 17·96 |
| | | | | | Average of Two Dogs after complete Removal of the Large Intestine. | | | | | | |
| 4 | 4 | 0·801 | 100 | 0·124 | 15·69 | 0·479 | 62·11 | 0·180 | 19·31 | 0·025 | 3·84 |
| 5 | 3 | 0·715 | 100 | 0·116 | 16·20 | 0·413 | 59·73 | 0·116 | 13·91 | 0·069 | 10·11 |

cent. of water was eliminated with 12 grams of fat, on increasing the fat to 62 grams only 67·87 per cent. of water is eliminated.

In the dog in which the large intestine was entirely removed, dog 4 with 9·71 grams of fat, the fæces contained 76·59, but on increasing the fat to 29·71 grams the percentage of water increased to 79 grams instead of falling as in normal dogs.

The effect of removal of the large intestine on the breaking up of fat in the alimentary canal was next investigated (Table XX).

In the above table the quantities of neutral fat, free fat acids, fat acids as soaps and cholesterin are given, and their percentage composition taking the total ether extract as 100. It is seen in all the dogs that the quantity of free fat acids is very much greater than that of the neutral fat; the quantity of fat acids as soaps and neutral fat correspond very much in percentage composition.

As far as the total quantity is concerned, that varies with the diet, but on the whole is comparable, so that one can conclude that the removal of the large intestine has no action in stopping either the breaking up of fat or the formation of soaps in the alimentary canal.

When we turn to the cholesterin, however, we find that there is a difference. The normal dog 1 excreted 0·154 gram and 2 only 0·061 gram of cholesterin. After partial removal of the large intestine the quantity of cholesterin corresponded very much with the normal dog 1, being 0·145 gram, while in the case of both dogs, when the large intestine was entirely removed, the quantity of cholesterin was very much less (in dog 4 only 0·025 gram, and in dog 5 0·069 gram); so that one may consider that the removal of the large intestine causes a decrease in the quantity of cholesterin, and this is probably explained by the loss of so much secreting surface in the intestine as would occur when the large intestine is removed.

The colouring matter in the fæces was also investigated in the normal dogs, and found in all cases to contain no bile pigment but marked quantities of urobilin, while in the case of the dogs in which the large intestine had been removed this was not always the case.

As far as the contents of the intestine or its walls are concerned the animals were killed with chloroform, and the presence of urobilin looked for throughout the intestine. In the normal dogs in the great majority of cases no trace of urobilin could be detected above the ileo-cæcal valve. The contents of the small intestine only gave the bile reaction.

In dog 5 the small intestine having been joined 6 cm. from the anus, a slight urobilin reaction was obtained as high as 35 cm. from the anus, the bile reaction as far as 78 cm. In this case evidently urobilin formation was taking place in the small intestine comparatively speaking high up.

In dog 4 only 4 cm. of rectum was left, and only in this part was

any urobilin reaction obtained, so that in this case as in normal dogs only the large intestine formed urobilin.

The next step in the investigation was to see the influence of diet and removal of the large intestine on the sulphates in the urine.

In Table XXVI it is seen that on increasing the fat in the diet, as the quantity of nitrogen decreases in the urine the quantity of total sulphates do the same; with 12 grams of fat a dog eliminating 0·637 gram of sulphates, with 32 grams of fat 0·544 gram, and with 62 grams only 0·521 gram. On the other hand it is seen that this steady increase in the quantity of fat accompanied by the decrease in the sulphates, is not due to a diminution in the quantity of aromatic sulphates, but of the alkaline sulphates, the aromatic sulphates remaining throughout practically the same, 0·064 gram. In consequence of the decrease in the alkaline sulphates the ratio A to B is decreased, so if one only referred to the ratio one would believe that there was an increase in the intestinal putrefaction, while on the other hand in reality there is no increase; if anything a decrease, as brought out in dogs 1 and 2, where the aromatic sulphates are not increased but, if anything, decreased.

In dog 3, in which the large intestine was partially removed, the influence of fat in the diet on the sulphates is the same as in normal dogs, and the quantity of aromatic sulphates corresponds with that found in normal dogs, so that one can say that there is no increase or decrease in intestinal putrefaction caused oy partial removal of the large intestine.

In dog 4 after complete removal of the large intestine it was seen the quantity of total sulphates corresponds with that found in normal dogs, only there is a marked decrease in the quantity of aromatic sulphates to half the normal. In consequence of this the ratio is very much increased, and, both by the ratio, increase, and the total decrease in aromatic sulphates, one sees clearly that intestinal putrefaction is very much diminished.

In dog 5 the same is brought out, but not to such a great extent, and, as is already seen, dog 5 was an exception as far as the urobilin was found to occur in the small intestine some way up.

In conclusion we may say that this research has led to some interesting results. That, as far as the large intestine influences the absorption of food stuffs, it has no action whatever on the carbohydrates of the diet, but its absence causes a marked decrease in the absorption of proteids from 93 per cent. to 84 per cent. The fat, on the other hand, is absorbed in practically the normal amounts, and it is found that the breaking up of fat continues the same when the large intestine is absent. The water of the fæces is increased in total quantity, although the percentage of water increases with an increased fat diet, instead of decreasing as in normal dogs. The total quantity of fæces is also

Table XXVI.—The Alkaline and Aromatic Sulphates. The Average Results of Normal Dogs as compared with Partial and Complete Removal of the Large Intestine, showing the Influence of an Increased Quantity of Fat in a fixed Proteid and Carbohydrate Diet. No. 1 and No. 2, Normal Dogs; No. 3, Partial Removal of Large Intestine; and Nos. 4 and 5, Complete Removal of the Large Intestine.

| No. | Duration of observation. | Diet. | | Quantity. | N. | Total. | Sulphates. | | A : B. |
|---|---|---|---|---|---|---|---|---|---|
| | | N. | Fat. | | | | Alkaline A. | Aromatic B. | |
| | days. | grams. | grams. | c.c. | grams. | gram. | gram. | gram. | Ratio. |
| 1*a* | 4 | 4·82 | 12·04 | 118 | 4·457 | 0·637 | 0·573 | 0·064 | 9 : 1 |
| *b* | 4 | 4·82 | 32·04 | 89 | 3·573 | 0·544 | 0·479 | 0·065 | 8 : 1 |
| *c* | 4 | 4·82 | 62·04 | 70 | 3·362 | 0·521 | 0·465 | 0·056 | 6·5 : 1 |
| 2*a* | 4 | 8·00 | 15·20 | 119 | 6·127 | 0·662 | 0·558 | 0·076 | 7 : 1 |
| *b* | 4 | 8·00 | 15·20 | 108 | 5·815 | 0·679 | 0·597 | 0·082 | 7 : 1 |
| *c* | 3 | 8·00 | 65·19 | 74 | 3·858 | 0·445 | 0·377 | 0·066 | 6 : 1 |
| 3*a* | 5 | 6·05 | 11·73 | 172 | 5·596 | 0·768 | 0·685 | 0·072 | 9 : 1 |
| *b* | 4 | 6·05 | 36·73 | 169 | 4·991 | 0·727 | 0·649 | 0·077 | 8 : 1 |
| *c* | 4 | 6·05 | 51·73 | 112 | 4·680 | 0·681 | 0·615 | 0·066 | 9 : 1 |
| 4*a* | 4 | 6·80 | 9·71 | 191 | 4·445 | 0·598 | 0·582 | 0·034 | 17 : 1 |
| *b* | 3 | 6·80 | 9·71 | 341 | 4·374 | 0·584 | 0·588 | 0·026 | 22 : 1 |
| *c* | 4 | 6·80 | 29·71 | 207 | 3·243 | 0·437 | 0·405 | 0·031 | 13 : 1 |
| *d* | 3 | 6·80 | 29·71 | 247 | 3·510 | 0·468 | 0·432 | 0·035 | 12 : 1 |
| 5*a* | 5 | 6·26 | 11·55 | 93 | 4·376 | 0·444 | 0·382 | 0·052 | 7 : 1 |
| *b* | 3 | 6·26 | 41·55 | 88 | 2·526 | 0·232 | 0·201 | 0·031 | 6·6 : 1 |

increased on the same diet as that in the normal dogs, and the cholesterin is decreased.

That the formation of urobilin in the fæces is diminished in the absence of the large intestine; the sulphates vary the same as the normal as regards those combined with the alkalis, while those combined with the aromatic substances are markedly diminished, showing that intestinal putrefaction is decreased.

## "Further Observations concerning the Relation of the Toxin and Anti-Toxin of Snake-Venom."

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The discrepancy between the quantities of anti-venene required to neutralise a given dose of venom when they are (1) previously mixed outside the body, and (2) simultaneously injected under the skin in different parts of the body, has been drawn attention to by Fraser and myself. My experience coincides with Fraser's* upon this point, viz., that it requires at least 10—20 times as much anti-venene to counteract a given dose of venom when they are injected separately, but at the same time, as is necessary to effect this if they are mixed together prior to injection.

Sometimes, however, the quantity necessary by simultaneous but separate injection may be much greater; in one of Fraser's experiments 1000 times as great.† Moreover, there is no constant ratio between the amounts necessary under the two conditions, as will be seen from the experiments tabulated below (Series A). In this series, experiments 1—4, in which increasing doses of venom were employed, show that 0·5 c.c. of the particular sample of serum used was more than adequate to prevent a fatal result when previously mixed for fifteen minutes at temperature 13° C. with 0·5 c.c. of a solution containing 0·0001 gram of the venom per c.c. As 0·00003 gram per kilo was found to be the minimal fatal dose of this poison, one may be sure that under these conditions 0·5 c.c. of the serum is adequate to neutralise more than 0·00002 gram of the venom, that is, 0·00005 gram *minus* one fatal dose.

In experiments 5—12, 0·00005 gram of venom per kilo. was injected in each case, and increasing amounts of serum separately, but at the same time. Under these conditions, every quantity less than 8 c.c., that is, sixteen times as much as is fully adequate to prevent any symptoms when brought directly into contact with the poison before

* 'Nature,' April 23, 1896.

† *Loc. cit.*, p. 594.