# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES


No. 11: THE COMPARATIVE VALUE OF LARD AND BUTTER IN GROWTH, BY CASIMIR FUNK and ARCHIBALD BRUCE MACALLUM

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# University of Toronto Studies

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# STUDIES ON GROWTH.

## III. THE COMPARATIVE VALUE OF LARD AND BUTTER FAT IN GROWTH.

BY CASIMIR FUNK AND ARCHIBALD BRUCE MACALLUM.*

*(From the General Memorial Hospital, Harriman Research Laboratory, Roosevelt Hospital, New York, and the Department of Pathological Chemistry, University of Toronto.)*

(Received for publication, August 3, 1916.)

In our first communication[1] of this series, we advanced the opinion that we were dealing with a problem very similar to, if not identical with, beri-beri. Our main objective was to ascertain the simplest dietary conditions necessary to enable a young rat to reach maturity. Subsequently we found[2] that artificial diets containing butter, without yeast or similar vitamine-containing substances, are insufficient to promote growth in young rats; and at that time the question whether butter could be replaced by lard with the same ultimate success was left open. We have carried out experiments of longer duration, employing both lard and butter as the fat fraction of these diets, and submit results which enable us to form a more definite opinion as to the relative value of these two fats.

Our experience demonstrates that there are wide variations depending on the constitution of the individual rats. Every rat taken indiscriminately is not suitable for this class of work. As a matter of fact in experiments carried out in Toronto 80 per cent of the rats purchased from dealers were rejected on account of physical defects not apparent before the initiation of the experiment. A second complication is a diminished resistance to infection, which follows the use of all artificial diets. The meager knowledge we possess of the pathological conditions

* Senior Fellow in Medical Research.

[1] Funk, C., and Macallum, A. B., Jr., *Z. physiol. Chem.*, 1914, xcii, 13.

[2] Funk and Macallum, *J. Biol. Chem.*, 1915, xxiii, 413.

in rats may lead to a condemnation of the diet; whereas actually the condition could be remedied without change of diet, if we were able to recognize its nature. As an example of this, rats on artificial diets frequently contract an eye infection which can be treated with a certain degree of success by an application of a few drops of zinc sulfate solution. If untreated this condition is accompanied by loss in weight, becomes acute, and terminates fatally.

The first series of experiments were carried out on diets containing lard as the fat component, and dried powdered yeast. Rats on this diet grew normally for 60 to 90 days, but eventually displayed symptoms (bleeding from the eyes, nose, and ears, petechiæ and hemorrhages under the skin of the tail) which might be regarded as scorbutic. This terminated fatally if no change of diet was effected. When moist yeast was substituted for the dried preparation the rats could be kept for 150 days and attained approximately adult size. Autolyzed yeast was equally efficient in this respect. Similar results were obtained on addition of orange juice to the drinking water, although orange juice itself has neither growth-promoting nor maintaining properties, unless supplemented by yeast.

Diets in which butter partially or wholly replaces lard have a slight superiority over those containing lard, which is more than can be explained by the antiscorbutic properties of the butter. Rats on yeast and butter diets often show the eye affection regarded by most of the investigators as characteristic of dietary deficiencies, and we are convinced that none of the artificial diets so far investigated can be compared with a normal dietary in its efficiency for growth. This deficiency introduces an additional complication and must be taken into account in subsequent investigations.

## EXPERIMENTAL.

The methods of preparing the diets were very much the same as those described in our earlier publications. The experiments varied slightly as to their conditions in New York and Toronto but the ultimate results were identical. The charts and tables are representative of the different groups of experiments.

*Experiment I.*

Rats 49, 50, 51, and 52 (Fig. 1) were kept on diets containing dried yeast and lard for about 68 days. At that time a deficiency was noticed which, in previous experiments, led to the death of all the rats and could not be corrected by a larger supply of dried yeast. Then fresh moist pressed yeast was substituted, the deficiency disappeared, and the rats attained approximately adult weight.

A second series, Rats 61 and 62 (Fig. 2), were placed on diets containing lard for 44 days, being changed to a diet of butter and dried yeast after this period. No increment in growth was noticed as the result of this change.

In the butter experiments Rats 53 and 54 (Fig. 1) have also shown a marked improvement on changing the yeast from the dried to the moist form, more especially as regards their external appearance. Rats 47 and 48 (Fig. 2) were kept on diets with butter and dried yeast and these have also developed symptoms which persisted when the diets were substituted by lard and autolyzed yeast. Rats 59 and 60 (Fig. 2), exceptionally healthy specimens, were kept on butter-containing diet for 44 days and then changed[3] to diet with lard and autolyzed yeast, for a longer period than was indicated in the chart, without the rate of growth being modified. On several occasions rats showing deficiency on a lard-containing diet were placed on a butter diet with the hope of relieving the symptoms. The improvement which resulted from this change was only temporary and several rats died after being kept 30 days on butter.

In all the experiments a marked improvement resulted when a diet of a different composition was given or even from a fresh preparation of the same diet. This might indicate that the diets lose part of their nutritive value when stored for lengthy periods.

[3] Encircled numbers on Fig. 2 indicate the point at which that diet was begun.

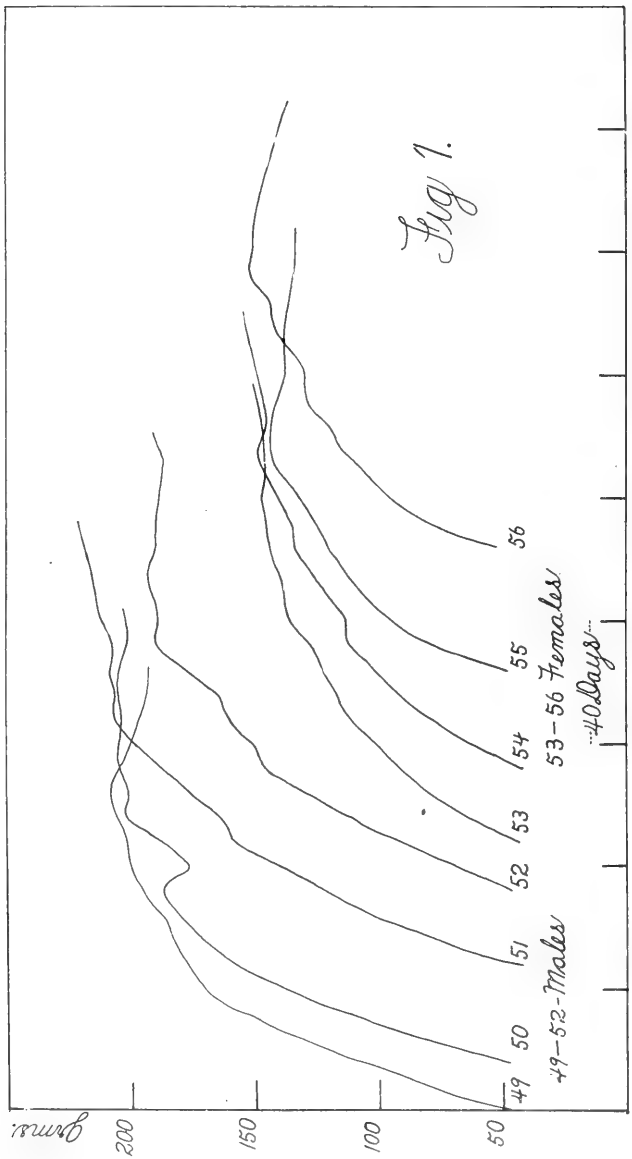


FIG. 1. Rats 49, 50, 51, and 52 were kept on diets containing lard and dried yeast. The animals recovered when moist yeast was substituted for dried yeast. Rats 53 and 54 were kept on butter and dried yeast. Here also a marked recovery was noticed on changing to the wet form of yeast. Rats 55 and 56 were kept on a diet containing casein purified according to the method of McCollum; no advantage of the use of this method is noticeable.

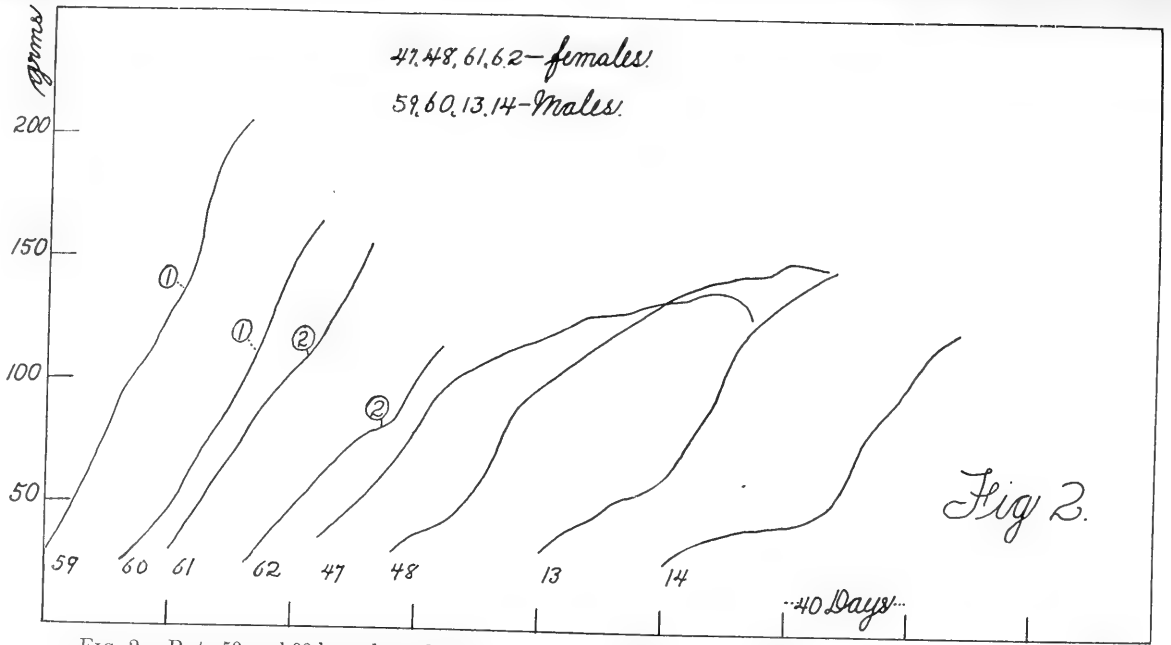


FIG. 2. Rats 59 and 60 have been kept on a diet of butter and dried yeast, which diet was then changed at the point indicated in the curve to lard and autolyzed yeast without any effect on the rate of growth. Rats 61 and 62 were kept on lard and then changed to butter without any effect on growth. Rats 47 and 48 were kept on butter and dried yeast and developed signs of food deficiency which persisted when the diet was changed to lard and autolyzed yeast. Rats 13 and 14 were kept on a diet containing casein which had been autoclaved. The rats failed to grow on this diet but recovered when 1 cc. of orange juice was added, which indicates that this deficiency was not due to chemical changes in the casein.

*Diets (Gm.).*

| | 1. | 2. | 3. | 4. | 5. |
|---|---|---|---|---|---|
| Casein | 22 | 22 | 22 | 22 | 22 |
| Sugar | 10 | 10 | 10 | 10 | 10 |
| Starch | 30 | 27 | 27 | 29 | 27 |
| Lard | 30 | 30 | 30 | 30 | 30 |
| Salt | 3 | 3 | 6 | 6 | 6 |
| Agar | 2 | 2 | 2 | 2 | 2 |
| Yeast (dry) | 3 | 6 | 3 | | |
| Yeast (moist) equal to | | | | 1 of dry yeast. | 3 of dry yeast. |

Rats 49 and 50. Males.
0– 68 days Diet 2
69– 98 " " 4
99–150 " " 5

Rats 51 and 52. Males.
0– 52 days Diet 1
53– 68 " " 3
69– 98 " " 4
99–150 " " 5

| Days. | Weight. | | Average food intake per day. | Weight. | | Average food intake per day. |
|---|---|---|---|---|---|---|
| | 49. | 50. | | 51. | 52. | |
| | *gm.* | *gm.* | *calories* | *gm.* | *gm.* | *calories* |
| 0 | 44 | 46 | | 41 | 46 | |
| 4 | 55 | 58 | 77.4 | 62 | 55 | 78.2 |
| 8 | 81 | 85 | 92.4 | 72 | 65 | 81.3 |
| 12 | 96 | 100 | 103.2 | 91 | 82 | 67.2 |
| 16 | 104 | 110 | 105.7 | 102 | 91 | 97.1 |
| 20 | 119 | 124 | 110.3 | 110 | 100 | 102.2 |
| 24 | 132 | 137 | 110.5 | 119 | 110 | 106.8 |
| 28 | 146 | 150 | 110.0 | 132 | 120 | 100.3 |
| 32 | 150 | 158 | 111.0 | 138 | 128 | 100.8 |
| 36 | 162 | 163 | 109.3 | 148 | 138 | 112.2 |
| 40 | 170 | 174 | 114.6 | 159 | 147 | 116.2 |
| 44 | 173 | 176 | 114.3 | 160 | 150 | 115.2 |
| 48 | 177 | 180 | 111.1 | 163 | 150 | 117.5 |
| 52 | 178 | 184 | 95.4 | 170 | 158 | 108.6 |
| 56 | 182 | 188 | 110.8 | 176 | 161 | 120.3 |
| 60 | 185 | 191 | 103.8 | 178 | 163 | 106.6 |
| 64 | 186 | 176 | 78.7 | 175 | 164 | 96.9 |
| 68 | 184 | 182 | 90.0 | 192 | 167 | 120.7 |
| 76 | 199 | 200 | 115.9 | | | 116.3 |
| 84 | 201 | 201 | 98.5 | 206 | 190 | 108.5 |
| 100 | 207 | 205 | 85.0 | 209 | 192 | 91.0 |
| 120 | 200 | 205 | 105.7 | 210 | 190 | 103.7 |
| 140 | 193 | 201 | 76.3 | 216 | 186 | 85.4 |
| 150 | 192 | 203 | 75.8 | 221 | 190 | 67.4 |

*Diets (Gm.).*

| | 1. | 2. | 3. | 4. |
|---|---|---|---|---|
| Casein | 22 | 22 | 22 | 22 |
| Sugar | 10 | 10 | 10 | 10 |
| Starch | 30 | 27 | 29 | 27 |
| Butter fat | 18 | 18 | 18 | 18 |
| Lard | 12 | 12 | 12 | 12 |
| Salt | 3 | 3 | 6 | 6 |
| Agar | 2 | 2 | 2 | 2 |
| Yeast (dry) | 3 | 6 | | |
| Yeast (moist) equal to | | | 1 of dry yeast. | 3 of dry yeast. |

Rats 53 and 54. Females.

0–52 days Diet 1
53–68 " " 2
69–100 " " 3
101–150 " " 4

| Days. | Weight. | | Average daily food intake. |
|---|---|---|---|
| | 53. | 54. | |
| | *gm.* | *gm.* | *calories* |
| 0 | 43 | 41 | |
| 4 | 54 | 55 | 74.3 |
| 8 | 64 | 63 | 66.2 |
| 12 | 78 | 75 | 72.0 |
| 16 | 80 | 80 | 77.0 |
| 20 | 85 | 87 | 73.5 |
| 24 | 90 | 92 | 76.8 |
| 28 | 96 | 95 | 81.1 |
| 32 | 100 | 101 | 79.2 |
| 36 | 106 | 106 | 88.9 |
| 40 | 112 | 112 | 87.3 |
| 48 | 112 | 112 | 87.2 |
| 56 | 119 | 116 | 83.7 |
| 64 | 123 | 126 | 95.2 |
| 84 | 138 | 135 | 79.8 |
| 100 | 144 | 146 | 76.5 |
| 120 | 149 | 147 | 79.2 |
| 150 | 147 | 153 | 92.8 |

*Diets (Gm.)*

| | 1. | 2. | 3. |
|---|---|---|---|
| Casein | 22 | 22 | 22 |
| Sugar | 10 | 10 | 10 |
| Starch | 28 | 30 | 23 |
| Butter | 30 | 30 | |
| Lard | | | 30 |
| Agar | 2 | 2 | 2 |
| Salts | 2 | 2 | 2 |
| $NaHCO_3$ | 1 | 1 | 1 |
| Yeast (dry) | | 3 | |
| Yeast (autolyzed) equal to | 1.5 of dry yeast. | | 3 of dry yeast. |

Rats 47 and 48. Females.
0– 16 days Diet 1.
16–123 " " 2.
123–140 " " 3.

| Days. | Weight. | | Average daily food intake. |
|---|---|---|---|
| | 47. | 48. | |
| | *gm.* | *gm.* | *gm.* |
| 0 | 36.5 | 31 | |
| 4 | 41 | 34 | 6.2 |
| 8 | 45.5 | 39 | 9.5 |
| 12 | 51.5 | 43 | 9.1 |
| 16 | 54.5 | 42.5 | 9.1 |
| 20 | 58 | 45 | 10.0 |
| 24 | 67.5 | 50.5 | 11.2 |
| 28 | 76 | 56.5 | 12.1 |
| 32 | 85 | 66 | 14.1 |
| 36 | 91 | 78 | 13.6 |
| 40 | 99 | 91 | 14.2 |
| 44 | 102 | 93 | 12.5 |
| 48 | 105 | 98 | 13.5 |
| 52 | 108 | 100.5 | 14.5 |
| 56 | 111 | 107 | 14.3 |
| 60 | 114.5 | 113.5 | 15.0 |
| 64 | 115.5 | 115 | 16.9 |
| 68 | 119 | 122 | 15.2 |
| 72 | 119 | 120 | 14.0 |
| 76 | 121.5 | 125.5 | 15.3 |
| 80 | 124.5 | 129.5 | 15.3 |
| 84 | 127 | 134.5 | 17.1 |
| 88 | 128 | 134.5 | 14.6 |
| 92 | 126.5 | 135 | 14.1 |
| 96 | 128.5 | 139 | 16.1 |
| 100 | 130.5 | 141 | 14.7 |
| 108 | 134 | 144.5 | 16.6 |
| 116 | 133 | 145 | 16.9 |
| 124 | 135 | 146.5 | 16.6 |
| 132 | 139.5 | 154 | 14.6 |
| 140 | 128 | 149.5 | 14.0 |

*Diets (Gm.).*

| | 1. | 2. |
|---|---|---|
| Casein | 22 | 22 |
| Starch | 23 | 30 |
| Sugar | 10 | 10 |
| Butter | | 30 |
| Lard | 30 | |
| Agar | 2 | 2 |
| Salts | 2 | 2 |
| $NaHCO_3$ | 1 | 1 |
| Yeast (dry) | | 3 |
| Yeast (autolyzed) equal to | 3 of dry yeast. | |

Rats 59 and 60. Males.
0–44 days Diet 2.
45–64 " " 1.

Rats 61 and 62. Females.
0–44 days Diet 1.
45–64 " " 2.

| Days. | Weight. | | Average daily food intake. | Days. | Weight. | | Average daily food intake. |
|---|---|---|---|---|---|---|---|
| | 59. | 60. | | | 61. | 62. | |
| | *gm.* | *gm.* | *gm.* | | *gm.* | *gm.* | *gm.* |
| 0 | 30.5 | 25 | 6.7 | 0 | 30.5 | 25.5 | 6.6 |
| 4 | 40 | 31 | 8.2 | 4 | 38 | 32 | 9.3 |
| 8 | 47.5 | 35.5 | 9.7 | 8 | 46.5 | 38 | 9.3 |
| 12 | 59 | 42.5 | 11.2 | 12 | 55.5 | 43 | 10.7 |
| 16 | 70 | 48 | 13.8 | 16 | 62.5 | 48 | 12.5 |
| 20 | 81.5 | 58.5 | 14.8 | 20 | 71.5 | 55 | 12.6 |
| 24 | 94 | 68 | 16.2 | 24 | 80.5 | 61.5 | 14.6 |
| 28 | 106 | 75.5 | 17.2 | 28 | 87.5 | 67 | 14.2 |
| 32 | 114 | 82.5 | 20.1 | 32 | 93.5 | 71 | 16.1 |
| 36 | 127.5 | 90 | 20.8 | 36 | 99.5 | 76 | 14.9 |
| 40 | 139.5 | 100.5 | 23.8 | 40 | 105 | 80.5 | 14.2 |
| 44 | 155 | 113 | 24.9 | 44 | 111.5 | 84.5 | 14.2 |
| 48 | 167 | 124 | 23.7 | 48 | 115 | 83 | 16.6 |
| 52 | 180 | 138 | 21.8 | 52 | 123 | 96 | 17.6 |
| 56 | 192 | 150 | 22.0 | 56 | 132 | 100 | 15.2 |
| 60 | 199 | 157 | 21.1 | 60 | 142 | 104.5 | 17.1 |
| 64 | 205 | 165 | 21.1 | 64 | 156.5 | 115 | |

## *Experiment II.*

The casein preparation used in this series was purified by washing, following the method of McCollum and Davis.[4] In

[4] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxiii, 231.

this paper the authors claim that purification of casein by boiling with alcohol destroys some of the amino-acids and results in loss of its nutritive properties. The results in this case (Rats 55 and 56, Fig. 1) were identical with those which were obtained with casein purified by extraction with hot alcohol. This latter method was used in purifying the casein in the first experiment.

*Diets (Gm.).*

| | 1. | 2. | 2. |
|---|---|---|---|
| Casein (McCollum) | 22 | 22 | 22 |
| Sugar | 10 | 10 | 10 |
| Starch | 27 | 29 | 27 |
| Lard | 30 | 30 | 30 |
| Salt | 3 | 6 | 6 |
| Agar | 2 | 2 | 2 |
| Yeast (dry) | 6 | | |
| Yeast (moist) equal to | | 1 of dry yeast. | 3 of dry yeast. |

Rats 55 and 56. Females.
0– 59 days Diet 1.
60– 87 " " 2.
88–140 " " 3.

| Days. | Weight. | | Average daily food intake. |
|---|---|---|---|
| | 55. | 56. | |
| | *gm.* | *gm.* | *calories* |
| 0 | 48 | 52 | |
| 4 | 67 | 71 | 75.1 |
| 8 | 80 | 82 | 92.2 |
| 12 | 89 | 91 | 88.4 |
| 16 | 96 | 96 | 77.2 |
| 20 | 104 | 102 | 86.0 |
| 24 | 107 | 104 | 74.9 |
| 28 | 109 | 109 | 94.3 |
| 32 | 115 | 116 | 79.8 |
| 40 | 120 | 120 | 88.4 |
| 60 | 140 | 133 | 86.3 |
| 80 | 144 | 143 | 78.4 |
| 100 | 136 | 150 | 79.9 |
| 140 | 133 | 136 | 61.9 |

Another series, of which Rats 13 and 14 (Fig. 2) are representatives, received casein which had been autoclaved for 1 hour at 15 pounds' pressure, according to McCollum and Davis. On this diet the rats failed to grow, but after 28 days 1 cc. of fresh orange juice was added, and normal growth was resumed. It seems probable that the impaired value of heated casein is not due so much to the destruction of amino-acids as to the loss of its anti-scorbutic properties.

*Diets (Gm.)*

| | 1. | 2. |
|---|---|---|
| Casein | 22 | 22 |
| Sugar | 10 | 10 |
| Starch | 30 | 29 |
| Lard | 30 | 30 |
| Salts | 3 | 3 |
| Agar | 2 | 2 |
| Yeast (dry) | 3 | 4 |

Rats 13 and 14. Males.
0– 14 days Diet 1.
15– 96 " " 2.
28–100 " 1 cc. orange juice.

| Days. | Weight. | | Average daily food intake. |
|---|---|---|---|
| | 13. | 14. | |
| | *gm.* | *gm.* | *gm.* |
| 0 | 33.3 | 27.2 | |
| 4 | 40.1 | 33.7 | 6.2 |
| 8 | 41.4 | 37.1 | 6.0 |
| 12 | 44.6 | 39.8 | 7.1 |
| 16 | 46.4 | 39.4 | 7.9 |
| 20 | 50.6 | 41.4 | 7.2 |
| 24 | 54.3 | 42.0 | 8.1 |
| 28 | 54.6 | 41.5 | 6.5 |
| 32 | 55.1 | 42.0 | 4.7 |
| 36 | 63.1 | 47.0 | 8.2 |
| 40 | 62.1 | 44.0 | 6.8 |
| 44 | 70.8 | 46.2 | 8.4 |
| 48 | 77.1 | 46.2 | 10.6 |
| 52 | 87.1 | 51.8 | 12.2 |

Rats 13 and 14. Males—*Continued.*

| Days. | Weight. | | Average daily food intake. |
|---|---|---|---|
| | 13. | 14. | |
| | *gm.* | *gm.* | *gm.* |
| 56 | 93.0 | 54.6 | 12.4 |
| 60 | 102.0 | 64.2 | 12.9 |
| 64 | 119.3 | 78.6 | 15.6 |
| 68 | 126.0 | 89.3 | 15.1 |
| 72 | 130.0 | 91.6 | 15.4 |
| 76 | 130.9 | 98.6 | 14.9 |
| 80 | 137.4 | 103.8 | 15.1 |
| 84 | 140.0 | 112.4 | 14.8 |
| 88 | 144.7 | 117.0 | 11.2 |
| 92 | 145.0 | 119.8 | 14.2 |
| 96 | 147.0 | 123.0 | 11.4 |

## SUMMARY.

The failure of rats to grow on a lard and yeast diet is partially due to the development of scorbutic symptoms. These can be relieved to a marked degree by using moist instead of dried yeast and still more so by using moist yeast and butter. Even in the latter case the existing deficiencies are not entirely corrected, since many rats decline on this diet. Rats which fail on lard do not always recover on a diet containing butter. It seems also possible that yeast on account of its high content in purines, and perhaps other constituents, is not an ideal addition in experiments of long duration, even in spite of its marked growth-promoting power. The impaired nutritive value of heated casein does not seem to be due to destruction of amino-acids but to destruction of vitamines.

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*General Editor:* H. H. Langton, M.A.
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# STUDIES ON GROWTH.

## IV. THE ACTION OF YEAST FRACTIONS ON THE GROWTH OF RATS.

By CASIMIR FUNK and ARCHIBALD BRUCE MACALLUM.*

*(From the General Memorial Hospital, Harriman Research Laboratory, Roosevelt Hospital, New York, and the Department of Pathological Chemistry, University of Toronto.)*

(Received for publication, August 3, 1916.)

The close relationship existing between the beri-beri and growth problems suggests the possibility of a fractionation of the active substance along lines already used in the investigation of beri-beri. Accordingly phosphotungstic acid was selected for the first attempt to separate out a physiologically active fraction which would stimulate the growth of young rats. The experimental difficulties which have repeatedly been emphasized in the investigation of the beri-beri vitamine, due to instability of this substance, were also encountered in the study on growth. The physiological activity of the different fractions diminishes with each manipulation and both the problems of beri-beri and of growth will not be solved until more suitable methods for the isolation of vitamine are available.

The results obtained so far clearly indicate that the growth-promoting substance is analogous to and possibly identical with the beri-beri vitamine and can be almost entirely precipitated with phosphotungstic acid. Subsequent fractionation of the residue obtained from the decomposed precipitate with silver nitrate and also with silver nitrate and baryta has shown that the precipitate containing purine bases and the filtrate from the silver nitrate and baryta precipitation are entirely negative as to their growth-promoting action; whereas the substances pre-

* Senior Fellow in Medical Research.

cipitated with silver nitrate and baryta possess traces of the activity of the initial phosphotungstic acid precipitate. The experimental evidence indicates that considerably larger quantities of vitamines are necessary for stimulating growth than for curing beri-beri, and the losses occurring during fractionation are more apparent in the former than in the latter case. However, it must be admitted that while it is uncertain whether these two substances are chemically different, the results obtained do not exclude such a possibility. Lloyd's reagent, as recommended by Seidell,[1] has also been used as a precipitant without much success, as the rats on the filtrate have also shown increments in growth.

In the first instance autolyzed yeast was slightly acidified with hydrochloric acid and completely precipitated with phosphotungstic acid, carefully avoiding an excess of this reagent. After allowing the mixture to stand for 24 hours, the precipitate was collected on a Buchner funnel and repeatedly washed with a cold solution of phosphotungstic acid containing hydrochloric acid. The precipitate was then decomposed by the method described by Van Slyke,[2] with a mixture of amyl alcohol and ether and hydrochloric acid, only a small quantity of the precipitate remaining unchanged. After filtration of this small fraction the aqueous extract was evaporated *in vacuo* and the residue made up to a known volume and mixed in the diet in quantities calculated from the amount of autolyzed yeast necessary to promote growth. However, the quantity of this fraction had to be doubled and even tripled in order to obtain satisfactory results. The phosphotungstic acid filtrate was worked out in a similar way. This process offers the advantage that the yeast fraction is completely freed from substances soluble in lipoid solvents. The purine fraction was obtained from the phosphotungstic acid precipitate fraction by precipitation with silver nitrate and subsequent decomposition with sulfuretted hydrogen. The filtrate from the purine bases was precipitated with baryta in the usual way and the precipitate decomposed, freed from traces of baryta, evaporated, and the residue mixed with

[1] Seidell, A., *U. S. Public Health Report*, No. 325, 1916.
[2] Van Slyke, D. D., *J. Biol. Chem.*, 1915, xxii, 281.

the diet. The filtrate from the fraction containing vitamines was reprecipitated with phosphotungstic acid and the precipitate obtained after thorough washing, decomposed with amyl alcohol and ether. The results with the purine fraction and also with the silver nitrate-baryta filtrate are not included in this paper as they were entirely negative. The effect of the silver nitrate-baryta fraction was not sufficiently marked to encourage further investigation. The diet contained lard as the fat constituent, and 1 per cent sodium bicarbonate was added to neutralize the hydrochloric acid present in this fraction. Orange juice to the extent of 1 cc. a day was added to the drinking water to prevent the onset of scorbutic symptoms.

A large number of rats were kept on the above diets, especially on the phosphotungstic precipitate and filtrate and the records of only a few were selected for publication, in order to save space. Rats 9 and 10 were kept on phosphotungstic acid filtrate throughout the experiment. Rats 11 and 12 were changed after 34 days to the diet containing the phosphotungstic precipitate fraction which was followed by an improvement warranting the view that the growth-promoting substance is contained in this precipitate (Fig. 2 b).

*Diets (Gm.).*

| | 1. | 2. |
|---|---|---|
| Casein | 22 | 22 |
| Sugar | 10 | 10 |
| Starch | 23 | 23 |
| Lard | 30 | 30 |
| Agar | 2 | 2 |
| Salts | 2 | 2 |
| $NaHCO_3$ | 1 | 1 |
| Phosphotungstate precipitate | | 10 |
| " filtrate | 10 | |

Rat 9 (male) and Rat 10 (female).
0–96 days Diet 1.
28–100 " 1 cc. orange juice.

Rats 11 and 12. Males.
0–34 days Diet 1.
35–100 " " 2.
76–100 triple vitamine addition.

| Days. | Weight. | | Average daily food intake. | Days. | Weight. | | Average daily food intake. |
|---|---|---|---|---|---|---|---|
| | 9. | 10. | | | 11. | 12. | |
| | *gm.* | *gm.* | *gm.* | | *gm.* | *gm.* | *gm.* |
| 0 | 35.2 | 41.8 | | 0 | 37.9 | 34.2 | |
| 4 | 42.8 | 47.3 | 9.5 | 4 | 46.2 | 41.8 | 7.6 |
| 8 | 43.0 | 47.3 | 7.8 | 8 | 45.8 | 44.2 | 5.5 |
| 12 | 44.6 | 49.3 | 7.0 | 12 | 42.8 | 45.3 | 7.6 |
| 16 | 46.8 | 55.4 | 8.7 | 16 | 45.3 | 45.8 | 7.8 |
| 20 | 46.2 | 56.6 | 7.5 | 20 | 46.9 | 45.7 | 7.7 |
| 24 | 46.6 | 54.7 | 7.8 | 24 | 46.2 | 46.5 | 6.6 |
| 28 | 46.5 | 52.8 | 5.5 | 28 | 44.3 | 45.0 | 5.4 |
| 32 | 42.9 | 49.9 | 7.4 | 32 | 40.4 | 39.4 | 4.7 |
| 36 | 37.8 | 41.6 | 7.8 | 36 | 39.8 | 37.2 | 5.6 |
| 40 | 36.8 | 41.6 | 7.7 | 40 | 50.5 | 48.2 | 9.1 |
| 44 | | 41.8 | 6.6 | 44 | 52.2 | 50.0 | 8.9 |
| 48 | | 43.9 | 5.3 | 48 | 54.8 | 57.0 | 8.0 |
| 52 | | 44.0 | 5.3 | 52 | 58.0 | 60.7 | 8.7 |
| 56 | | 43.8 | 3.8 | 56 | 63.5 | 67.3 | 11.0 |
| 60 | | 41.6 | 3.5 | 60 | 66.3 | 71.0 | 12.4 |
| 64 | | 44.6 | 3.5 | 64 | 66.1 | 70.3 | 8.8 |
| 68 | | 41.0 | 3.2 | 68 | 70.6 | 75.7 | 11.1 |
| 72 | | 43.5 | 3.5 | 72 | 75.0 | 82.6 | 9.9 |
| 76 | | 42.9 | 3.4 | 76 | 76.0 | 84.8 | 11.9 |
| 80 | | 45.0 | 3.0 | 80 | 77.6 | 91.1 | 11.1 |
| 84 | | 42.2 | 3.1 | 84 | 75.0 | 85.2 | 9.6 |
| 88 | | 44.1 | 2.7 | 88 | 79.4 | 90.0 | 8.8 |
| 92 | | 43.0 | 3.2 | 92 | 78.5 | 90.9 | 4.4 |
| 96 | | 41.0 | 2.9 | 96 | 74.7 | 91.7 | 6.4 |
| 100 | | | | 100 | 70.0 | 76.3 | 6.4 |

A second series of experiments was carried out on pressed yeast which had been heated with 10 per cent sulfuric acid at 90–95° for 6 hours. The hydrolysate was filtered, diluted with an equal volume of water, and precipitated with phosphotungstic acid. After standing for 24 hours the precipitate was filtered at the pump and well washed with 5 per cent sulfuric acid.

The precipitate was decomposed in the ordinary way with baryta. The final solution, slightly acid, was neutralized with sodium carbonate, carefully avoiding an excess, distilled *in vacuo*, standardized, and definite quantities were added to the diet. The

filtrate of the phosphotungstic acid precipitation was treated in a similar way.

The diet containing the substances precipitated by phosphotungstic acid was fed to four rats (Rats 80 to 83, Fig. 1) and enabled them to double their original weight after 32 to 36 days. This is about double the time required when yeast is the source of vitamines and the depreciation is due to the fractionation with the precipitating reagent.

Two rats (84 and 85, Fig. 2 a) were fed the diet containing the residue from the phosphotungstic filtrate. After 28 days they had added only a third to their original weight and had all the external symptoms of an acute deficiency. Then the diet with the precipitate was substituted and in 11 days they rapidly doubled their original weight and presented a normal appearance.

*Diets (Gm.).*

| | 1. | 2. |
|---|---|---|
| Casein | 22 | 22 |
| Sugar | 10 | 10 |
| Starch | 24 | 24 |
| Fat (lard) | 30 | 30 |
| Salt | 6 | 6 |
| Agar | 2 | 2 |
| Residue phosphotungstic acid precipitate equal to | 6 | Dried yeast. |
| Residue phosphotungstic acid filtrate equal to | | 6 |

Rats 80, 81, and 82. Males.
Rat 83. Female.
0–36 days Diet 1.

| Days. | Weight. | | Average daily food intake. | Weight. | | Average daily food intake. |
|---|---|---|---|---|---|---|
| | 80. | 81. | | 82. | 83. | |
| | *gm.* | *gm.* | *calories* | *gm.* | *gm.* | *calories* |
| 0 | 24 | 25 | | 20 | 30 | |
| 4 | 31 | 33 | 29.8 | 23.5 | 37 | 27.8 |
| 8 | 36 | 40.5 | 33.5 | 28 | 43 | 32.8 |
| 12 | 36 | 43 | 35.7 | 29 | 45 | 33.3 |
| 16 | 38 | 44.5 | 40.5 | 29 | 48 | 40.5 |
| 20 | 40 | 48 | 28.6 | 31 | 51 | 38.3 |
| 24 | 42 | 49 | 30.1 | 34 | 54 | 30.2 |
| 28 | 44.5 | 52 | 27.8 | 38 | 57 | 35.0 |
| 32 | 46 | 54 | 28.7 | 40 | 59 | 27.4 |
| 36 | 49 | 56 | 25.0 | 42.5 | 61 | 29.8 |

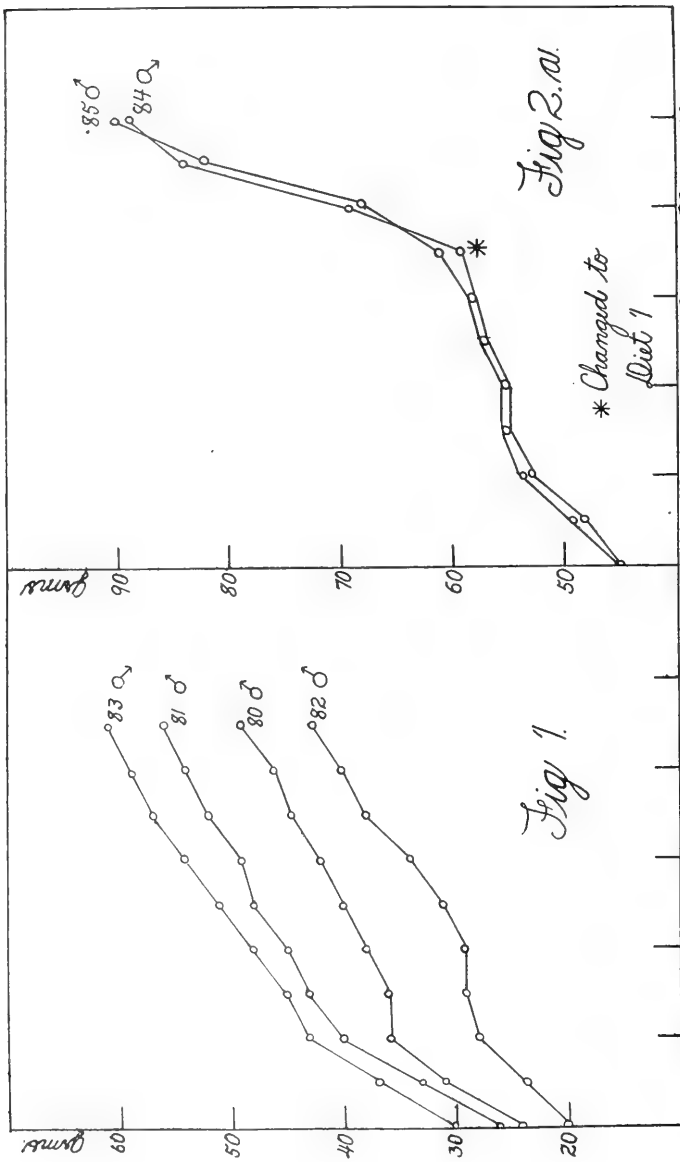


FIG. 1. Rats 80, 81, 82, and 83 were kept on a diet containing decomposed phosphotungstic acid precipitate from yeast.

FIG. 2 a. Rats 84 and 85 were kept first on a diet containing the residue from phosphotungstic filtrate of yeast. The marked deficiency was corrected when this addition was changed on the point marked on the chart to the corresponding precipitate.

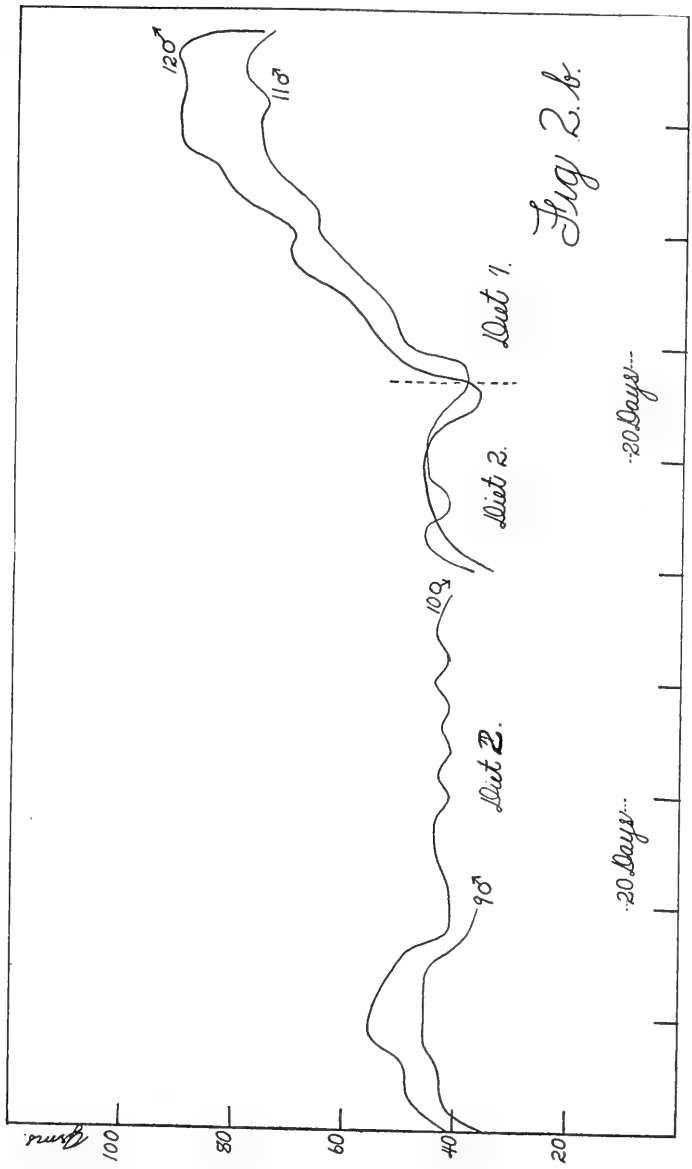


FIG. 2b. Rats 9 and 10 were kept on a diet containing phosphotungstic acid filtrate throughout the experiment. Rats 11 and 12 were changed to phosphotungstic precipitate at the point marked on the chart, with a marked improvement in growth and general appearance.

Rat 84. Female.
Rat 85. Male.
0–28 days Diet 2.
29–39 " " 1.

| Days. | Weight. | | Average daily food intake. |
|---|---|---|---|
| | 84. | 85. | |
| | *gm.* | *gm.* | *calories.* |
| 0 | 45 | 45 | |
| 4 | 48 | 49 | 28.6 |
| 8 | 53 | 54.5 | 46.3 |
| 12 | 55 | 55 | 42.9 |
| 16 | 55 | 55 | 35.9 |
| 20 | 57 | 57 | 50.9 |
| 24 | 58 | 58 | 48.6 |
| 28 | 59 | 61 | 47.9 |
| 32 | 69 | 68 | 54.6 |
| 36 | 84 | 82 | 70.3 |
| 39 | 89 | 90 | 66.3 |

SUMMARY.

The fractionation of yeast with phosphotungstic acid shows that the growth-promoting substance is carried down with the precipitate and a large part of its activity is lost during the fractionation. The instability of this substance when fractionated with silver salts presents greater difficulty than that experienced during the fractionation of the beri-beri vitamine. It seems possible that both these problems can only be solved when more adequate methods are available.

UNIVERSITY OF TORONTO STUDIES

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ON CHANGES IN THE GLOMERULI AND TUBULES OF THE KIDNEY ACCOMPANYING ACTIVITY, by PROFESSOR T. G. BRODIE and PROFESSOR J. J. MACKENZIE

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# CROONIAN LECTURE: *A New Conception of the Glomerular Function.*

By T. G. BRODIE, M.D., F.R.S., Professor of Physiology in the University of Toronto.

(Lecture delivered June 15, 1911,—MS. received December 9, 1912.)

[PLATE 26.]

I have chosen as the subject of this lecture the physiology of the kidney, and more particularly the mode of action of one part of it, namely the glomerulus. In 1906, at the meeting of the British Medical Association in Toronto, I brought forward a new conception of the action of this very characteristic portion of the renal apparatus, and since that time have been accumulating a considerable mass of evidence by the light of which my theory can be criticised.

Very shortly after the discovery of the main details of the structure of the kidney, Ludwig, basing his ideas upon the then known structure, put forward his well-known theory that the glomerulus was a filter, and since that time all discussions upon renal activity have centred round this theory because it offered an explanation of the mode of action of one part of the mechanism upon hydrodynamic principles. The necessary corollary following from this assumption of filtration is that a considerable degree of absorption must be effected as the dilute filtrate travels down the tubule, and how excessively great this must be was first pointed out by Heidenhain.

If we consider the results obtained by the earlier workers upon the kidney, very many of them appear sufficiently well explained by the Ludwig theory, but as in the course of years a far stricter examination of the theory was attempted, several observations were made which proved very difficult to explain, and in many cases it was necessary to make such extensive and often contradictory assumptions that it became increasingly difficult to accept the theory. Of recent years evidence has been obtained in many directions which in my opinion conclusively proves that the glomerulus is not a filtering surface. It is not my object to-day to discuss this point in any detail. I may refer to my lecture delivered before the Harvey Society in New York in December, 1909, where a short summary of the facts for and against filtration is given, or to the excellent paper by Magnus, in the 'Handbuch der Biochemie,' where it is discussed *in extenso*. It will be sufficient for my present purpose if I indicate the chief reasons which led me

to conclude that the idea of filtration at the glomerular surface must be abandoned.

Perhaps the most striking piece of evidence is derived from the consideration of the concentration and constitution of the urines obtained during extremely free secretion. The evidence is quite clear that the main bulk of the water secreted by the kidney undoubtedly comes from the glomeruli. Hence the more rapid the flow of fluid from the kidney the more closely must that fluid resemble in constitution the fluid discharged from the glomeruli, since a much shorter time is then allowed to the cells of the tubules to modify it by absorption or secretion, and if filtration is the active process in the glomeruli this fluid ought to approximate more and more closely in composition to the blood plasma so far as the salts, urea and all constituents of the plasma other than proteins are concerned. But the dilute urine secreted after drinking copious amounts of lager beer,* or of water,† shows a constitution in salts widely different from that of the blood. Considering only the total concentration, as estimated by the depression of the freezing point, it is quite easy to obtain a urine with $\Delta = -0{\cdot}1^\circ$ C., and one as low as $-0{\cdot}075^\circ$ C. has been recorded.‡ To effect a change in concentration so extensive as this denotes, by filtration through a semipermeable membrane, would necessitate a pressure difference on the two sides of the membrane of at least 4000 mm. Hg, a pressure difference utterly out of comparison with the blood-pressure. Therefore to make such a result accord with the filtration theory, it becomes necessary to assume a most extensive reabsorption of the salts and other substances of small molecular size, a reabsorption on such an extensive scale and at such a rate as is, I think, entirely out of the question.

If, in the second place, we investigate the correlation between the blood flow and the rate of secretion, we find that while there is a general correspondence, in that increased urine flow is usually accompanied by increased blood flow, this is by no means a universal rule.§ I have frequently observed in kidneys in which there was at the start a fairly free blood flow and but slow urine secretion, a copious diuresis to come on without any change in the blood flow. Indeed on no less than five occasions I have seen a distinct decrease in the blood flow to occur as the diuresis commenced, and moreover in these experiments the volume of the kidney actually increased. In every direction we find that the urine flow does not vary strictly with the blood flow nor

* Dreser, 'Arch. Exp. Path.,' 1892, vol. 29, p. 303.

† Macallum and Benson, 'Journ. Biol. Chem.,' 1909, vol. 6, p. 87.

‡ Macallum and Benson, *loc. cit.*

§ *Cf.* Gottlieb and Magnus, 'Arch. Exp. Path.,' 1901, vol. 45, p. 223.

with the blood-pressure, as should be the case were filtration the essential factor in determining the volume of the urine discharged from the kidney.

In the third place we have very decisive evidence against the Ludwig theory in experiments designed to test the second assumption in that theory, namely that of reabsorption. If this is a process which occurs extensively within the tubules, and we bring into play any factor which favours reabsorption, we ought to effect a diminution in the volume of urine yielded by the kidney. Such a factor is an increase in hydrostatic pressure within the ureter, tending to prevent the outflow of urine. All that is necessary is to make the kidney discharge against a small pressure. The experiments carried out by most experimenters upon these lines have indeed yielded results which may be interpreted as indicating increased reabsorption. But we may urge as a general criticism against such results that the degree of decrease of urine flow is surprisingly small when we remember how essential it is according to Ludwig's theory to assume that reabsorption is excessively free. The kidney working against even a small hydrostatic pressure ought to show far greater reabsorption than was actually obtained. But the whole idea of reabsorption as an active process in the formation of urine has been completely disproved by Miss Cullis and myself,* for we were able to prove that decrease in rate of the urine flow when a kidney was made to secrete against a pressure was only a universal result when the animal was under an anæsthetic, and that if the animal were pithed and the experiment then performed in the absence of an anæsthetic, the kidney working against a small pressure always excreted more salt and usually more water than the opposite kidney.† The action of a pressure then tends to excite the kidney to greater activity, a result which entirely disproves the possibility of reabsorption being an extensive factor in the normal formation of urine.

Yet another point which militates greatly against the idea that the glomerulus is a filter is the behaviour of the kidney after temporary asphyxiation. If the renal artery be clamped for one minute and then released, the kidney does not at once begin to secrete, although the blood flow returns at once. It is only after a variable, but usually considerable delay that the kidney restarts, and at first the urine flow is very slow, only gradually returning to a rate comparable to the initial flow. If the artery has been clamped for any length of time the urine first collected after

* Brodie and Cullis, 'Journ. of Physiol.,' 1906, vol. 34, p. 224.

† Subsequent to these experiments I have found that, under the same conditions, the blood flow through the kidney is not altered by the small rise in ureter pressure employed in our experiments.

the re-establishment of the circulation contains protein, casts, even hæmoglobin, indicating considerable damage to the renal epithelium, either of the tubules or of the glomeruli or of both. But even if the glomerular epithelium be damaged it is inconceivable that this should temporarily abolish all the filtering properties it formerly possessed, and it is just as difficult to understand why the recovery of its power to filter should occur so gradually when the asphyxiation is arrested.

Let us next turn to the evidence that has been sought in favour of Ludwig's theory from experiments upon the maximum ureter pressure. One of the earliest attempts to associate the formation of urine directly with the blood-pressure was a measurement of the maximum height to which the kidney could force the urine up a vertical tube. As is well known, in the case of the salivary gland, the gland can secrete water to a pressure exceeding that of the blood in the carotid artery, a clear indication that a new force, viz., one exerted by the salivary gland cells, is at play in producing the result. But in the case of the kidney the result is very different. For the maximum ureter pressure always lies below the aortic blood-pressure, and usually some 30–40 mm. Hg below that pressure. The results were therefore interpreted by supporters of the filtration theory as indicating that as soon as the pressure within Bowman's capsule reached a point some 30 mm. Hg below the glomerular blood-pressure, filtration ceased, and Starling* explained the difference between the aortic pressure and the maximum ureter pressure as being the pressure difference necessary for the separation of the blood proteins from plasma, for he estimated the osmotic pressure of the blood protein at that amount. It has since been shown, however, that the protein osmotic pressure is certainly much less than this. Moreover Starling failed to allow for a loss of pressure between the aorta and the glomerular capillaries. Without doubt the loss of pressure between these points is less than in the case of ordinary capillaries, for the resistance in the kidney arterioles when dilated is certainly much less than at most points on the systemic circulation. As I shall show later, the maximum ureter pressure as ordinarily taken is a measure of the blood-pressure in the glomerular capillaries.

But a still more difficult problem is offered to those accepting the filtration theory in explaining these experiments. As was first pointed out by Heidenhain,† upon the Ludwig theory the maximum ureter pressure should be that pressure which just suffices to effect complete reabsorption of all the glomerular filtrate. Upon the theory we are to imagine an absorbing surface, capable of absorbing water, chlorides, urea and most of the bodies filtered in

* Starling, 'Journ. of Physiol.,' 1899, vol. 24, p. 317.

† Heidenhain, 'Hermann's Hdb.,' vol. 5, p. 327.

urine at a very fast rate. Such an absorbing surface would be influenced, as indeed is usually assumed by the supporters of Ludwig's theory, by a rise in pressure of the fluid at the surface. It then becomes very difficult to explain how the ureter pressure could ever be driven so high as is usually observed, especially when we remember that the rise in pressure can be effected with great rapidity.

Yet another result obtained in these experiments upon maximum ureter pressure is very significant. I have found that the maximum ureter pressure is practically the same whether the kidney be made to secrete a moderate amount of urine or a very large quantity. If reabsorption be a very active process, then the maximum ureter pressure in the latter case ought to be distinctly higher than in the former. As a matter of fact, it is not.

Taking everything into account, therefore, I have very grave doubts as to the occurrence of reabsorption in the tubules, and I am sure, if it does take place, that it is insignificant in comparison to that demanded by Ludwig's theory.

*The Function of the Glomerulus.*

Arriving then at the conclusion that the filtration theory was incorrect, I came back once more to the old problem: How are we to explain the very peculiar and characteristic structure shown by the glomerulus? I finally hit upon the idea that it was simply a means of utilising the blood-pressure for setting up a pressure head sufficiently great to drive the urine secreted at the glomerular surface down the tubule. To express this idea I term the glomerulus a propulsor. As is abundantly proved, the main volume of the water of the urine is secreted into the capsule of the glomerulus. To drive it from the capsule down the tubule requires a definite pressure-head. Whence is this pressure head derived? My view is that the intraglomerular blood-pressure is transmitted directly through the thin-walled glomerular loops to the fluid which has been secreted into the capsule, and thus a pressure is communicated to the fluid sufficient to force it down the tubule. To test this view, let us imagine that a certain amount of fluid has accumulated within Bowman's capsule. The problem then becomes: How is that fluid discharged down the tubule? If we know the number, length and lumina of the tubules, and the total amount of fluid leaving the kidney within a given time, it becomes easy to calculate the pressure-head which must have existed within each capsule in order to drive the fluid out of the kidney. It is simply an application of Poisseuille's law. I therefore performed two experiments upon the following lines. An active diuresis was established in an anæsthetised dog, and the rate at which urine was being discharged from one of the kidneys was determined. The pedicle of the kidney was

then ligatured and the kidney fixed entire in 10-per-cent. formalin solution. After fixation the whole kidney was cut into slices each about 7 mm. thick. The medulla was carefully separated from the cortex, and the latter collected and weighed. Next three small pieces of the cortex, selected from different regions of the kidney, were weighed separately. These were imbedded in paraffin and serial sections mounted. The sections were about 8$\mu$ thick. The next point was to determine the number of sections through which a single glomerulus extended. For this purpose ten glomeruli were followed through the series, and the mean number of sections through which one glomerulus ran thus ascertained. Lastly the total number of glomeruli in each section was counted, and the total number for all sections, divided by the average number for a single glomerulus, gave the total number of glomeruli present in that block of cortex. Similar calculations were made from each of the other two pieces. Then, knowing the weights of the three pieces and the total weight of the cortex, the number of glomeruli in the whole kidney was obtained.* The first dog weighed 11 kgrm., its right kidney weighed 34·5 grm., and the total number of glomeruli was 142,000. A kidney of a second dog, weighing a little over 8 krgm., contained 125,000 glomeruli.

Employing a different method, Peter† calculated the number of glomeruli in the dog's kidney as 300,000. He does not give the weight of the kidney, nor does the method he employed appear to me comparable in accuracy with that above described. I have not been able to find any further record of enumerations of the glomeruli in the dog's kidney, and I wish to acknowledge my great indebtedness to Miss M. G. Thackrah for carrying out this very tedious piece of work.

Measurements of the lumina of the tubules in their several parts were now made, as also approximate estimates of the lengths of the tubules based upon the measurements of Peter.

The average results obtained from these measurements in the case of the first kidney were :—

| | Length. cm. | Diameter. $\mu$. |
|---|---|---|
| Proximal convoluted tubule ..... | 1·2 | 12 |
| Loop of Henle— | | |
| Descending limb ..... | 0·9 | 10 |
| Ascending limb ..... | 0·9 | 9 |
| Distal convoluted tubule ..... | 0·2 | 18 |
| Collecting tubule..... | 2·2 | 16 |

* This is practically the method originally adopted by Huschke in 1828 ('Isis,' vol. 21, p. 550).

† Peter, 'Verhandl. D. Anat. Ges., Würzburg,' 1907, p. 120.

The diuresis at the time the kidney pedicle was ligatured was 1 c.c. per minute.

From the formula for the flow of liquids along narrow tubes

$p = 8l\eta/\pi r^4$ times flow in cubic centimetres per second dynes per square centimetre,

where $l$ is the length of the tubule in centimetres,

$\eta$ is the coefficient of viscosity, and

$r$ is the radius of the tube in centimetres.

Taking $\eta$ as $719 \times 10^{-5}$, the coefficient of viscosity of water at 35° C., we have

$$p = \frac{8 \times 719 \times 10^{-5}}{\pi} \cdot \frac{1}{60} \cdot \frac{1}{142000} \cdot 10^{16} \cdot \frac{l}{r^4} \text{ dynes per square centimetre,}$$

$r$ being now expressed in microns; or

$$p = \frac{8 \times 719 \times 7}{22 \times 6 \times 142 \times 1333 \cdot 2} \cdot 10^7 \cdot \frac{l}{r^4} \text{ mm. Hg.} = 1 \cdot 611 \times 10^4 \times \frac{l}{r^4} \text{ mm. Hg.}$$

Consequently, for a flow of 1 c.c. per minute,

| | $\mu$. | mm. Hg. |
|---|---|---|
| $p$ per centimetre of tubule, when $r$ = | 4·5, | = 39·29, |
| $r$ = | 5, | = 25·78, |
| $r$ = | 6, | = 12·43, |
| $r$ = | 8, | = 3·93, |
| $r$ = | 9, | = 2·46. |

Hence pressure-head required for—

| | | mm. Hg. |
|---|---|---|
| Proximal convoluted tubule..... | = 1·2 × 12·43 = | 14·916 |
| Loop of Henle— | | |
| Descending limb ..... | = 0·9 × 25·78 = | 23·212 |
| Ascending limb..... | = 0·9 × 39·29 = | 35·361 |
| Distal convoluted tubule ..... | = 0·2 × 2·46 = | 0·492 |
| Collecting tubule ..... | = 2·2 × 3·93 = | 8·646 |
| Total pressure-head..... | | 82·627 |

In the case of the second kidney, with 125,000 tubules, the measurements were:—

| | Length. cm. | Diameter. $\mu$. |
|---|---|---|
| Proximal convoluted tubule..... | 1·0 | 12 |
| Loop of Henle— | | |
| Descending limb ..... | 0·8 | 10 |
| Ascending limb ..... | 0·8 | 10 |
| Distal convoluted tubule ..... | 0·2 | 18 |
| Collecting tubule ..... | 2·0 | 8 |

And, with a diuresis of 0·85 c.c. per minute, the pressure-head required works out to 74·1 mm. Hg.

I do not wish to lay too great a stress upon the actual pressure-head thus obtained, for the possible errors in the measurements are many. It is, for instance, impossible to obtain anything but an approximation to the lengths of the successive portions of the tubule, and also the measurements of their lumina can only be approximate, for they are undoubtedly altered during fixation. Also I have supposed all the tubules to have equal lumina, and have neglected to take into account those tubules which were at rest. To obtain the total pressure within Bowman's capsule a factor for the velocity head should be added to the pressure-head already calculated, but it is so small that we may omit it. (The mean velocity within the narrowest portion of the tubule amounts to about 1 mm. per second.)

The important point is that during an active diuresis a pressure-head of the order of 80 mm Hg. may be needed within Bowman's capsule to drive the fluid secreted there down the tubule.

The mean aortic blood-pressure in the first experiment was 120 mm. Hg, and in the second 115 mm. If we allow 30–35 mm. Hg as the loss of pressure-head between the aorta and the glomerular capillaries when the afferent glomerular vessels are dilated, the blood-pressure within the capillary loops would amount to 90–85 mm. Hg in the first experiment, and 85–80 mm. in the second. Hence, on these figures, practically the whole of the blood pressure-head is required to set up a pressure-head in the fluid within the capsule sufficient to drive the secreted fluid down the tubule. Bearing in mind that the estimates given are only approximate, I conclude that the pressure-head within Bowman's capsule only differs from the pressure-head within the glomerular loops by the pressure required to stretch the walls of the loops. This latter probably does not amount to more than one or two millimetres of mercury.

If in the light of these arguments we criticise once more the assumptions made by Ludwig's theory, we see that that theory becomes less tenable than ever. In the first place, when the kidney is secreting water at its fastest rate, the pressure difference available for filtration is reduced to a minimum. At lower rates of secretion, of course, a pressure difference might be available. In the second place, the assumption must be made that the volume of water discharged from the glomerulus is from 30 to 70 times greater than the volume of water entering the pelvis of the kidney. Hence a very much greater pressure-head would be required to drive that fluid down the tubule, though not 30 to 70 times greater than the pressure required to drive a volume equal to that of the discharged urine, since the fluid has to be driven only as far as the absorbing surface. But as the absorbing surface would have to be taken as extending at least to the end of the ascending limb of

the loop of Henle, *i.e.* along considerably more than one-half of the whole tubule, and the whole length of the narrowest part of the tubule, the pressure-head required would be enormous, certainly many times greater than the glomerular blood-pressure. We should, therefore, be compelled to ascribe to the cells secreting the water the power of setting up a very high hydrostatic pressure, and all the evidence is strongly against any such view. A pressure within Bowman's capsule greater than the blood-pressure would at once lead to the closure of the glomerular loops and arrest of the circulation. This is the main reason why neither the cells of Bowman's capsule, nor these covering the glomerular tufts, nor those of the convoluted tubule, possess the power of setting up a hydrostatic pressure.

The quantity of energy imparted by the blood to the glomerular secretion is only a small percentage of its total amount. Thus if V be the minute volume of blood flowing through the glomerulus, and $v$ the minute volume of glomerular secretion, then V c.c. of blood enter the glomerular capsule, and $V-v$ c.c. leave it. If $p$ be the pressure-head in the glomerular loops, the pressure energy of the blood entering is $Vp$, and that of the blood leaving is $(V-v)p$. The pressure energy communicated to the glomerular secretion is $vp$, and the ratio of this to the total pressure energy of the blood as it enters is $v/V$. In the dog's kidney V may have any value from 200 to 600 c.c., and $v$ from 1 to 2 c.c. at the height of a diuresis. Thus the pressure energy given up by the blood lies somewhere between 1 and 0·16 per cent. of its total pressure energy.

### *Histological Evidence.*

In the next instance the test applied was that of microscopical examination of the kidney after varying degrees of activity. If during diuresis fluid is being forced at a considerable pressure from Bowman's capsule down the tubule, evidences of the action of this pressure should be indicated by changes both in the glomerulus and in the tubule. It is very remarkable that throughout the literature the accounts of changes in the glomerulus following activity are so scanty, and many authors state that no changes whatever are to be found (*e.g.* Lamy and Mayer). Mackenzie and I therefore examined a number of kidneys excised after diuresis had been induced under various conditions, and found that decided changes are produced in the glomerulus and tubule. We further found abundant evidence proving that the tubules have been subjected to a high internal pressure. The full details of these changes are given in a separate paper.* The general results are as follows:—

On comparing a resting kidney with one that has been thrown into activity

* *Vide* p. 593.

by the injection of any diuretic which causes a free flow of water, the differences between both glomeruli and convoluted tubules are of the most striking character. These differences are illustrated in figs. 1 and 2, which show the changes in the cortex under a low magnification. The important points are the following:—In an active kidney the glomeruli are always separated from the capsules, and usually there is a considerable accumulation of fluid in this position. The capsule is always rounded, whereas in the resting kidney the capsule lies in contact with the glomerulus, and the whole structure is usually irregularly polyhedral in shape. In an active kidney, in contradistinction to the resting, the individual loops of the glomerulus are frequently separated from one another and stand out clearly. The glomerulus also has a very characteristic vacuolated appearance, due, we think, to dilated capillaries, from which the red blood corpuscles have in some way or other been removed or destroyed, possibly *post mortem*. When examining two such kidneys under a low power of magnification the contrast is most striking. In the resting kidney the glomeruli are far from conspicuous, and have to be sought for. In the active kidney, on the other hand, they stand out at once as the most conspicuous objects in the field of view.

The changes in the tubules are just as striking. Whereas a resting proximal convoluted tubule possesses no lumen, one in activity has a large lumen. This is true both of the proximal and the distal tubules. Moreover, in the resting kidney the tubules are very much twisted on themselves and form very complicated foldings, whilst in the active kidney the appearances indicate that the tubule is as far as possible straightened out. All these several points prove quite clearly that the tubules have been subjected to some high fluid pressure from within.

The changes accompanying activity are strikingly emphasised when we measure the diameters of these several structures. In the case of the glomeruli and capsules, in addition to measurements in diameters at right angles to one another, approximate calculations of their volumes were also made.

In one experiment which we may take as typical we obtained the following results:—

| | Resting. | After activity. |
|---|---|---|
| Volume of capsule..... ..... | 83* | 220 |
| „ glomerulus ..... | 80 | 111 |
| „ fluid in capsule ..... | 3 | 109 |

* These figures can be converted into cubic millimetres by multiplying them by $4{\cdot}2 \times 10^{-6}$.

The differences are therefore very great. The capacity of Bowman's capsule in the active kidney is nearly three times that of the capsule in the resting kidney, chiefly on account of the big accumulation of fluid within the capsule.

The volume of the glomerulus has also increased, though only by 40 per cent. Such measurements prove, therefore, that both the glomerulus and the capsule of Bowman are extensible structures, and that a considerable volume of fluid accumulates in the capsule during activity.

In drawing deductions from these measurements, full attention must be paid to possible alterations occurring after the kidney is excised. To obviate change as far as possible in these experiments, the artery, vein and ureter were ligatured close to the hilum at the instant the experiment was to be stopped, using a single coarse ligature. The kidney was then excised, rapidly weighed, and placed at once in the formalin fixative. If active diuresis were in progress, the kidney at the moment of ligature was hard and tense, but within a few seconds after application of the ligature became quite soft, chiefly on account of escape of blood through the Capsule. We found it impossible to avoid this. The question therefore arises: Does this fall of tension within the kidney substance involve a change in distribution of the fluid contained within the tubule and capsule? It is possible, for instance, that fluid is forced back from the distended tubule into the capsule. Possibly this may be the cause of some of the increase in volume of the capsule seen in our experiments, but the changes are too great to be wholly, or even largely, explicable in this way. There is yet another *post-mortem* change we think possible, viz., that before the fixative has time to penetrate and reach the glomeruli, the cells forming the loops die and permit osmotic effects to take place through them between the fluid in the capsule and the blood. Fluid would pass into the blood, and we think it possible that this fluid is so low in salinity as to lake some of the corpuscles, thus producing the vacuolated appearance described above.

In the same experiment the measurements of the diameters of the proximal and distal convoluted tubules and of their lumina were as follows:—

| | Resting. | Active. |
|---|---|---|
| | $\mu$. | $\mu$. |
| Proximal convoluted tubule— | | |
| Transverse diameter ..... | 44·0 | 43·0 |
| Lumen, diameter ..... | 0·0 | 19·4 |
| Distal convoluted tubule— | | |
| Transverse diameter ..... | 25·4 | 31·8 |
| Lumen, diameter..... | 11·0 | 21·8 |

This is fairly typical of the results obtained in all our experiments. We found it to be practically a universal rule that the external diameter of the proximal convoluted tubule remained unaltered, or showed but a slight increase or decrease. The marked change during activity is the production of a big lumen within the proximal tubule. The idea given by an examination of the sections is that the loops of the convoluted tubule have been opened out and stretched in length. They are in nearly all instances circular in outline, and invariably, as just stated, there is a very wide lumen. The distal convoluted tubule in contradistinction is nearly always increased in diameter in the active state, and the lumen greatly increased, often doubled, although this tubule has invariably a rather large lumen even in the resting kidney.

We have not yet carried out a sufficient number of measurements of the remaining portions of the tubule to warrant us making any decided statement as to the changes they undergo. It is clear that the limbs of the loop of Henle are both distended, and often the collecting tubules show very distinct expansion.

The next modification in our experiments consisted in comparing the two kidneys after active diuresis, one kidney having been previously stripped of its Capsule.

The kidney is very characteristically enclosed in a strong and practically inextensible Capsule*, and my view of the meaning of the glomerulus offers an explanation of that fact. As fluid is secreted into Bowman's capsule by the epithelium covering the glomerular loops, and possibly also by the epithelium of the capsule, the blood-pressure acting within the glomerular loops is transmitted directly to that fluid and through it to the wall of Bowman's capsule. This latter, as we have seen, is extensible and might be ruptured if the distension were carried too far. Again, fluid is at once forced into the convoluted tubule, and that also might be ruptured if overdistended. To prevent any dangerous overdistension the whole of the structures are enclosed in a firm Capsule. That this distension does take place on activity is amply proved in a variety of ways. Firstly, as shown above, the histological appearances demonstrate it. Secondly, if in an experiment we excise one kidney at the commencement, then excite diuresis, and at its height ligature the pedicle of the other kidney to prevent escape of urine from the tubules, and we then weigh the two kidneys, the latter often shows an increase in weight amounting to about 30 per cent. This increase in weight is not due to blood, for on excision the blood escapes more readily from such a kidney than from a

* In order to avoid confusion between the Capsule of the kidney and Bowman's capsule, I will when referring to the former distinguish it by a capital.

resting kidney. In the third place I have often observed the following changes during the course of an oncometric experiment, viz., a large increase in the volume of the kidney, a free flow of urine, but a decrease in the rate of blood flow through the kidney. Here the plethysmographic increase is due to an accumulation of urine within the capsules and tubules. Lastly, if we examine a kidney at the height of a diuresis we always find it very hard and tense. The Capsule is distended to its fullest degree. If we attempt to make such a kidney expand still further by temporarily clamping the vein we fail completely. We see then that some of the tension set up by the blood-pressure in the glomeruli is transmitted through the capsule wall and the walls of the tubules to the general renal tissues. How much pressure is thus transmitted depends upon the resistance to distension offered by Bowman's capsule and the walls of the convoluted tubules. Their structure, particularly that of the capsule, indicates that they probably offer a fairly considerable resistance. We could get an estimate of this by finding the difference between the blood-pressure in the glomeruli and the general tension of the kidney substance within its Capsule. I made some attempts to measure this latter during active diuresis, but at present have not obtained any very accurate results. As far as they go they indicate a tension of about 40 mm. Hg.

If this be the true meaning of the kidney Capsule then, if we remove it before exciting diuresis, the kidney ought to expand still further as compared to the intact one, and the amount of that further expansion should depend upon the general rigidity of the kidney substance and the amount of connective tissue it contains. Our experiments proved this to be the case. The weight of such a kidney compared to one with the Capsule untouched was always greater, especially in the rabbit's kidney. In the cat there are a number of incomplete septa running transversely towards the hilum, and on active diuresis the kidney substance bulges notably between these, giving the appearance of constricted grooves in the bottom of which veins run. This relatively greater increase in volume of the kidney as a whole is also found in the several parts of the tubule, and when we measured the tubules and glomeruli in such kidneys, the differences were very distinct. For instance, in one experiment the right kidney was untouched, and the left decapsulated. The following approximate volumes of the capsule and glomerulus after diuresis were obtained:—

| | R. | L. |
|---|---|---|
| Volume of capsule ..... | 205 | 257 |
| „ glomerus ..... | 128 | 151 |
| „ fluid ..... | 27 | 106 |

The diameter of the tubules was as follows:—

| | μ. | μ. |
|---|---|---|
| Proximal convoluted tubule— | | |
| External diameter | 44·8 | 48·0 |
| Lumen | 13·0 | 19·8 |
| Distal convoluted tubule— | | |
| External diameter | 33·2 | 39·2 |
| Lumen | 24·8 | 29·2 |

The expansion then is found in all parts, and is obviously brought about by a distending force acting within the tubules.

Yet another means of testing the theory which presented itself was to observe the effect of obstructing the exit of urine down the ureter. In the first set of experiments a diuresis was set up, and at its height the ureter on one side was suddenly clamped. Five to fifteen minutes later the two kidneys were exposed, their condition noted, and then the pedicles ligatured as close as possible to the hilum. The kidneys were then removed and weighed. As was to be expected, a kidney obstructed in this manner is very distended and tense within its Capsule. The weights found in one experiment in which the right kidney was obstructed at the height of diuresis, and the left secreting freely, were as follows:—

| | grm. |
|---|---|
| Weight of R. kidney | 15·5 |
| „ L. kidney | 13·5 |

The right kidney was very tense, appeared almost bloodless, and was distinctly lobulated. The left kidney was distinctly softer than the right and also more vascular. The approximate volumes of the capsules and glomeruli were:—

| | L. | R. |
|---|---|---|
| Volume of capsule | 86 | 146 |
| „ glomerulus | 69 | 89 |
| „ fluid | 17 | 57 |

The measurements of the tubules were:—

| | μ. | μ. |
|---|---|---|
| Proximal convoluted tubule— | | |
| External diameter | 39·2 | 39·4 |
| Lumen | 6·6 | 14·0 |
| Distal convoluted tubule— | | |
| External diameter | 22·8 | 28·2 |
| Lumen | 12·6 | 18·4 |

Lastly, in an experiment in which an obstructed kidney was compared to a decapsulated one we found that the former procedure produced more effect than decapsulation.

*Maximum Ureter Pressure.*—Another series of observations which receive a satisfactory explanation is that in which the maximum ureter pressure is measured. According to my theory, fluid should be forced out of the tubules only when the pressure within the ureter lies below the maximum glomerular blood-pressure. This of course assumes that the tubular epithelium in secreting does not set up any appreciable hydrostatic pressure. From this point of view the measurement of the maximum ureter pressure should be a means of determining the intraglomerular blood-pressure, always supposing that none of that pressure is taken up by the walls of the glomerular loops. Now the measurements of the maximum ureter pressure fit in perfectly with this conception. In an animal whose aortic blood-pressure is about 120 mm. Hg, the maximum ureter pressure is usually found to be somewhere between 80 mm. and 90 mm. Hg, that is, a loss of pressure-head of some 30 to 40 mm. Hg occurs between the aorta and the glomerular capillaries. This is distinctly less than is the case for most systemic vessels, and fits in well with our knowledge of the relatively wide and short path of the blood stream from the aorta to the glomerulus. We have only to recall how fast the blood may flow through the kidney to realise that the glomerular capillary pressure during activity must stand at a greater height than the ordinary systemic capillary pressure.

Let us then return to a general restatement of the whole problem. I have given evidence that the glomerulus, Bowman's capsule and certain parts of the tubules are elastic structures, and that their overdistension is prevented by the general inextensibility of the connective tissue framework and of the Capsule. Consequently as soon as fluid is secreted by the glomerular surface into the capsule, the glomerular capillary pressure comes into play, and some part of that pressure is transmitted through Bowman's capsule to the tubules immediately outside. Then as the secretion continues to accumulate, the kidney expands to fill the Capsule, and the pressure within the Capsule reaches its maximum. Hence we may regard the glomeruli as a number of expanding vascular tufts, lying within a space which cannot expand beyond a certain point, consequently the expansion of the glomeruli expels any fluid free to move outwards. It is as if we were dealing with a sponge work filled with fluid, and enclosed in a capsule which it completely fills. Distributed through the sponge are a number of elastic structures which can be expanded by a fluid pressure acting from within, their expansion necessarily compressing the sponge, *i.e.* expelling the fluid from between its interstices. This analogy

is of course incomplete, in that it takes no account of the tubular structure and the facts that the pressure is set up in the fluid within the tubules and that the walls of the tubules offer some resistance to expansion. The first effects of the glomerular pressure will therefore be to distend the capsule and the first convoluted tubule, *i.e.* to increase its lumen, thus offering less resistance to the flow of fluid along the tubule. In this distension the pulsation of the glomerular vessels is probably utilised. Also the more rapid the flow along the tubule the greater the pressure gradient, and the smaller the pressure transmitted through the walls of the tubules to the general kidney substance. We must therefore expect to find a distinct difference between the intratubular pressure and the intra-Capsular pressure, and while fluid is moving down the tubule the two could only be equal at the point where the tubule leaves that part of the kidney substance where the pressure is raised. This region is limited as we shall see by the branching arches of the renal vessels in the intermediate zone.

There is yet another feature of the renal structure and form which is capable of interpretation by this theory. This is the general shape of the mammalian kidney, so typical as to give its name to all structures in any way resembling it. The kidney is very typically constructed of a cortical mass enveloping a medullary portion. The blood-vessels form a set of arches between these two parts. My suggestion is that this arched system of vessels forms a more or less rigid base upon which the cortex lies. Consequently when, in activity, the pressure in the general renal tissue rises through the activity of the glomeruli it is restricted in the first instance to the cortex. The cortex, so to speak, becomes compressed between the rigid Capsule and the firmly distended arterial arches. From this general pressure the medullary portion is relieved, and it is a most significant fact that the loops of Henle lie within this region, where there is probably but little external pressure. Apparently, then, the difference in state between the tubules in the cortex and those in the medulla is that there is a high pressure on both internal and external surfaces of the tubules lying in the cortex, whereas in the medulla the pressure may be acting chiefly, possibly entirely, from the inner surface of the loops only. In this connection I have frequently observed the following most notable result:—If at the height of a diuresis whilst urine is flowing freely the ureter be ligatured, and after about 20 minutes the pedicle be tied off and the kidney removed, it will be found that the pelvis is widely distended with fluid, and usually the pyramid is compressed towards the cortex until it forms an almost insignificant structure projecting into the cavity of the pelvis. Histo-

logically the tubules within such a collapsed pyramid are observed to be flattened and empty.

It is possible that some or even all of this compression might be *post mortem*, but I think that it is *ante mortem*, since it is only found if sufficient time be allowed to lapse between the ligaturing of the ureter and the removal of the kidney. The longer the interval the more marked is the compression. I think the compression is produced in the following way :—After the ureter has been ligatured urine continues for a time to be expelled into the pelvis, and gradually the pressure there rises. Fluid will continue to be forced into the pelvis in gradually decreasing volume until the pressure reaches that of the glomerular capillary blood-pressure. The further distension of the pelvis and compression of the medulla is probably produced through the pulsatory variations of pressure in the cortex. The systolic pressure, by the expansion of the glomeruli and arteries, suddenly raises the tension throughout the whole cortex ; this expels a little of the fluid from the terminal portions of the tubules into the pelvis, whose pressure then becomes greater than diastolic pressure. As the pressure falls in diastole a point is reached at which the cortical pressure is below the pressure in the pelvis, that is below the pressure in the fluid contained within the loops of Henle and the collecting tubules. Accordingly these latter are emptied or partially emptied into the cortical tubules, while the lower ends of the collecting tubules are compressed and act as valves, preventing any return flow from the pelvis up the tubule. In this way more and more fluid is gradually collected within the pelvis at the expense of the medulla.

If, as I think is the case, we may divide the kidney substance into two parts, in one of which the whole tubule is exposed to a considerable pressure, both internal and external, while in the other region the pressure is largely within the tubule, the difference must have some important physiological meaning. It is most significant that the loops of Henle are carried down into this region of low external pressure. In different animals the loops of Henle show many diversities of form, more particularly in length, and it is certainly a striking fact that in some animals the major number of loops are short, and either lie completely within the cortex or only descend into the outermost portions of the medulla. It has been pointed out that the animals with very short loops are those which secrete a dilute urine, whilst those in which the loop penetrates far into the medulla secrete a concentrated urine. Hence it may be that this loop effects a certain amount of absorption, a function which would be aided by a pressure difference acting from within the tubule.

To test my theory further, and in the hope of gaining some evidence of the

respective activities of the different parts of the renal apparatus, another series of experiments was performed, in which the action of diuretics upon animals whose blood-pressure had been lowered by section of the spinal cord was tested. It was necessary to employ rabbits for these observations, since in both the cat and the dog the blood-pressure remains high enough after section of the cord to enable the kidney to secrete quite freely when a diuretic is administered. In the rabbit the blood-pressure falls to about 30 mm. Hg, and even though we injected large doses of saline and other diuretics we never obtained a single drop of urine from the kidneys. The plan of experiment therefore was to excise one kidney some 10 to 20 minutes after division of the spinal cord, then inject the diuretic to be studied, and half-an-hour later to remove the other kidney. In this way evidence was obtained indicating the point of action of various diuretics. Without going into the results in detail, I may state that the glomerulus is excited to secrete by most of the diuretics of the saline group. Thus activity was well marked after sodium sulphate, urea, or dextrose; it was excited also by caffeine, but completely absent after phloridzin. In the tubules the results were equally striking, especially in the case of phloridzin, and in a minor degree in the case of caffeine. In no instance was a large lumen produced, and the external diameters of the convoluted tubules were only slightly increased. The contents of the lumen consisted of fairly large secretion droplets, the droplets being enclosed in membranes which stained with Weigert's hæmatoxylin, and fairly well with eosin. These results were chiefly observed in the proximal convoluted tubule. With the low blood-pressure there was never the slightest indication of any marked distension of the tubule in any part of its course. The glomeruli were never found secreting very actively, but were always found separated from the capsular epithelium by a distinct though small accumulation of fluid.

An examination of the embryology of the renal tubule bears out the views I have expressed. Originally, the excreting apparatus was a long tubule opening at one end into the body cavity, and at the other on to the surface. This tubule was lined throughout by a ciliated epithelium, which provided the necessary motor mechanism for the expulsion of the secretion. Later, the glomerulus was developed from the dorsal wall of the body cavity and received a large and important blood supply from the aorta. Possibly its original function was to secrete a watery fluid into the body cavity, and this in some way served the renal tubule. The arrangement of its vessels as large loops projecting from the cœlomic wall, even at this early stage, tends to indicate that it was employed as a means of raising the fluid pressure within the

cœlom. In the next stage of development that part of the body cavity which contained the orifices of the renal tubules and the glomeruli became largely constricted off from the rest, and by means of imperfect septa the glomeruli also became partially separated from one another. This indicates that the function of the glomerulus has now been restricted almost solely to work in association with renal excretion. Later, this becomes entirely the case by the complete separation of that portion of the cœlom from the rest. Each glomerulus then works in conjunction with a renal tubule, but at first the number of the latter is largely in excess of the former. The material secreted at the glomerular surface is now conducted entirely to the tubule, as is also any formed by the isolated portion of the cœlomic endothelium. It is very significant that as soon as the relationship between glomerulus and tubule is completed the latter loses its cilia, only the cells of the neck of the tubule retaining them in some animals. This indicates that some other mechanism for the propulsion of fluid down the tubule has taken the place of the ciliary movement. This, according to my view, is the propulsive action of the glomerular capillary loops.

*Previous Work Bearing upon the Subject.*

L. Hill, in discussing the general distribution of pressure through a soft and yielding animal tissue, arrives at the conclusion that filtration is an impossible mechanism at the glomerular surface. With much that Hill expresses in his paper on "Filtration in the Living Organism,"* I am in complete agreement, but in several points I think he is incorrrect. Thus, he considers that the glomerular capillary pressure must be transmitted in undiminished amount throughout the whole renal tissue. This implies that the wall of Bowman's capsule is incapable of offering any resistance to extension, and similarly, too, for the walls of the tubule. Our measurements show, however, that while these structures expand, they offer resistance to expansion. They indicate that a higher pressure has been acting on the internal surface of the tubule than on the outer, and especially until a sufficient dilatation has been produced to make the kidney substance as a whole expand, and thus render the Capsule tense. From that point on, the tension in the kidney substance rapidly rises. I have found by measurements of the blood flow that at this point the blood flow falls, due, that is, to compression of the capillaries around the convoluted tubules and of the renal veins. The fact that the capillary system which originates this pressure consists of characteristic tufts which lie entirely within capsules is very significant. In certain forms of tubular nephritis, in which the

* 'Biochem. Journ.,' 1906, vol. 1, p. 55.

tubules are blocked or obliterated, and have been so for a considerable time, the capsules are often found distended to a volume even ten times greater than the normal volume. In these cases the glomerulus is collapsed and shrunken to a minute structure, which appears as a mere projection into the swollen capsules.

In my opinion, too, Hill does not allow a sufficient fall in pressure-head between the glomerular capillaries and the tubule capillaries. The efferent blood-vessel of the glomerulus is of small diameter and fairly long. Hence with the exceedingly rapid blood flow observed during diuresis, there must of necessity be a considerable pressure difference between these two capillary systems. I cannot, therefore, agree with Hill's statement: "The pressure of the secretion cannot be normally greater than the pressure *in* the veins, for otherwise the secretory pressure would compress the veins"; nor, again, with the statement: "The secretion moves onward, I take it, by phenomena of adsorption."

At about the same time Filehne and Biberfeld* reasoned that filtration at the glomerular surface was an impossibility, since there were no firm supporting structures capable of resisting any pressure. They, too, consider that the glomerular capillary pressure is at once transmitted through the whole renal substance, leaving no pressure difference available for filtration through the glomerular surface. While agreeing with them that but a very minute pressure difference can exist between the glomerular blood-pressure and the pressure of the secreted fluid within Bowman's capsule, I am in disaccordance with them, for reasons already stated, in their idea that the glomerular pressure is at once transmitted in undiminished amount to the general renal substance.

Shortly after I had expressed my views as to the work of the glomerulus, Lamy and Mayer† published a paper in which they suggested that the glomerulus by its pulsation acted as a kind of heart, and by its piston-like movements drove the liquid forward in the tubule, and favoured its discharge by overcoming the friction and the capillarity of the tubule. They do not consider that the glomerulus plays any important part in the secretion of water. If it secretes any at all, this is in their opinion quite a minor rôle. According to them the glomerulus performs mechanical work solely by virtue of its pulsation, and consequently their view differs widely from mine. I am, in the first place, in wide disagreement with them in that I consider that the main bulk of the water is secreted by the glomerular surface. There is abundant evidence to prove this. I need only refer to the work

* 'Pflüger's Archiv,' 1906, vol. 111, p. 1.

† 'Journ. de Physiol.,' 1906, vol. 7, p. 660.

of Miss Cullis upon secretion in the frog's kidney,* or to the results I have briefly described above upon secretion in the rabbit's kidney after division of the spinal cord. As is seen from what I have stated, the fact that the glomerulus pulsates has but little bearing, if any, upon its work in propelling the secreted water along the tubule. That pulsation is unimportant in the propulsor action of the glomerulus is borne out by the fact that the urine flows quite freely along the ureter of an excised kidney perfused with fluid at constant pressure, and if in these cases the perfusing fluid be of correct composition, the kidney presents at the end of the experiment appearances exactly comparable to those found by Mackenzie and myself after active diuresis in the intact animal. It is possible that pulsation may play a part in producing the primary dilatation of the convoluted tubule. In an artificial schema representing the glomerulus and tubule, I have found that the volume of fluid driven along the capillary tube by a pressure made to vary in imitation of the pulse variations is exactly the same as if a steady pressure at the mean height of the varying one is used. This indeed was to be expected from theoretical reasons. The value of a varying pressure only arises when the tubule along which the fluid is to be driven has first of all to be expanded.

In conclusion, then, we may summarise what I have said in the following way:—

The glomerulus is a secreting surface whose chief function is to secrete the main bulk of the water of the urine, but it is also thrown into activity by such substances as salts, urea, dextrose and caffeine. Its highly characteristic shape is to enable it to act as a means of setting up a pressure-head sufficient in amount to drive the secreted water down the long urinary tubule. The pressure originating from this is also transmitted in some degree through Bowman's capsule to the general tissues of the cortex, thereby exerting a pressure upon the external surfaces of all the tubules lying in the cortex. To what degree the pressure on the external surfaces of the convoluted and other tubules lies below the glomerular capillary pressure I am not yet able to state definitely. The fact that the convoluted tubules show such marked evidences of having been subjected to a high internal pressure certainly indicates a considerable diminution. I have also given reasons for believing that the general pressure conditions so typical of the cortex are non-existent in the medulla; there, apparently, the internal pressure acts upon the loops of Henle in undiminished amount, and must be supported either by the basement membrane of those tubules, or by the general tissue of the medulla itself. At present the former seems the more probable. Lastly I have given evidence attained by the application of yet another method, which enables us

* 'Journ. of Physiol.,' 1906, vol. 34, p. 250.

to determine from histological evidence the part of the urinary apparatus thrown into activity by the different urine exciting substances.

[*Addendum.*—Shortly after I delivered this lecture before the Royal Society, letters appeared in the 'Lancet' and the 'British Medical Journal' by Mr. Wm. Woods Smyth, claiming that his brother, Dr. A. W. Smyth, had over 30 years ago anticipated the views I now expressed. Dr. Smyth's views of the function of the kidney appeared in a pamphlet by Mr. John Gamgee, in the 'New Orleans Medical and Surgical Journal' for May, 1880, and were based upon microscopic examination of the kidney, and upon the fact that the kidney pulsated with each heart-beat. As far as I am aware, no reference to his views has ever appeared in the literature upon the kidney. They concerned the glomerulus and the circulation through the kidney. He denies the existence of any "connection between the capsule of the Malpighian body and the interior of a uriniferous tubule," and also "having observed that the hyaline membrane, enclosing each glomerule, was unprovided with epithelium, essential to every secreting structure, Dr. Smyth perceived that so delicate a sac would rupture, and the plexus be destroyed, if subjected to hydrostatic pressure, either during secretion or from accidental regurgitation." But the main point in relation to this lecture is his view of the mode of working of the glomerulus. This he describes in the following terms:—"Every heart-beat is attended by turgescence of the glomerule. The loops, from their position and form, must swell outward and inward in all directions, and, constricting the efferent vessel, momentarily impede the blood's exit. At each cardiac diastole, the arterial column sustaining the blood in its channel, the Malpighian loops recoil and fill the current in the secreting vascular rete. And this is Dr. Smyth's view of the special function of the Malpighian bodies. Their alternate turgescence constituting a 'rhythmic vascular impulse,' a uniform, safe, and sufficient expelling pressure is maintained on the coiled tubes, and, indeed, on the whole excreting structure of the kidney. Those acquainted with the laws which govern the flow of liquids can readily understand that the power required to maintain a circulation, beyond the coils of the glomerule, would be destroyed, if a mere physical transudation could occur through the loops, so well disposed to bring the very active pulsation to bear on the maintenance of a circulation."

"The unmistakable constriction of the efferent vessel, on the filling of each glomerule, causes an alternation between clearance of the tubuli and the flow of blood in the secreting vascular rete. The glomerules are filled during the heart's systole; the secreting rete is turgid during the heart's diastole."

Undoubtedly Dr. Smyth's conjecture was in the right direction, but his erroneous conclusion that Bowman's capsule did not open into the tubule, and the fact that he ascribed all the expelling power of the glomerulus to its pulsation, will indicate sufficiently the great divergence of his views from those I have expressed in my lecture.]

## DESCRIPTION OF PLATE.

Fig. 1.—Microphotograph of Cortex of Kidney of Cat, after period of rest, showing absence of lumen in convoluted tubules and irregular outline of glomeruli. ×120.

Fig. 2.—Microphotograph of Cortex of Kidney of Cat, after sulphate diuresis, showing widely dilated tubules and distended capsules, which are now rounded and contain much fluid. The glomeruli are larger than in the resting kidney, but not filling the capsules. ×120.

HARRISON AND SONS, Printers in Ordinary to His Majesty, St. Martin's Lane.

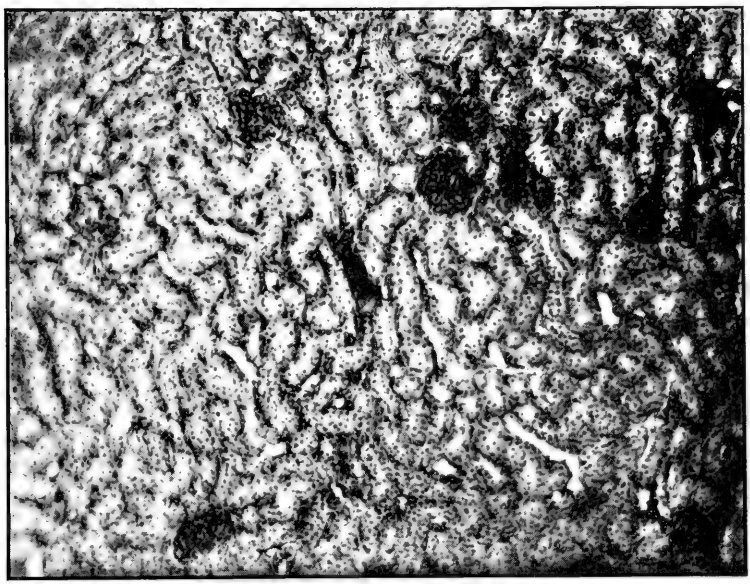

FIG. 1.

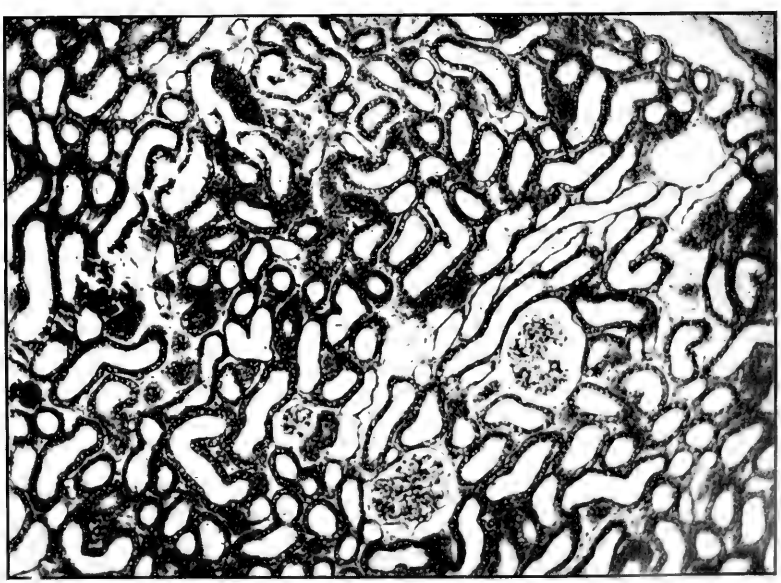

FIG. 2.

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# *On Changes in the Glomeruli and Tubules of the Kidney accompanying Activity.*

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[PLATE 27.]

The experiments described in this paper were designed to test the correctness of the view put forward by one of us,* namely, that the glomerulus is a propulsor. If this view be correct, the marked dilatation of the tubules, which is so prominent a feature in a kidney after active diuresis, is simply the expression of the forcible distension of the tubule from within, effected by the discharge of fluid from the glomerulus down the tubule, the active propelling and dilating force being the intraglomerular blood-pressure transmitted through the glomerular capillary cells and epithelium. As, however, the condition of the glomerulus after active secretion has not been made the subject of extensive observation, it seemed probable that a thorough study of the alterations in size and appearance of both tubule and glomerulus might give many points of importance in criticising the propulsion theory. Thus, if the capsule be free to expand, we may find it enlarged after active diuresis; and again, if the propulsive action of the glomerulus is complete and instantaneous, we should find the glomerulus filling Bowman's capsule completely under all conditions. But it was also possible that, after a very free secretion of water, there might be a considerable accumulation of fluid between the glomerulus and the capsule wall. We therefore measured the sizes of the capsules, the glomeruli and the tubules in kidneys, before and after diuresis had been set up under varying conditions. The more important of these states were:—

1. The kidney at rest.
2. The kidney secreting freely. This we term an "active free" kidney.
3. Decapsulated and secreting freely. This we term an "active decapsulated" kidney. The aim of the procedure was to test the explanation offered by the theory as to the meaning of the Capsule.†

* *Vide* Croonian Lecture, *supra*.

† As in the course of this paper we shall be referring constantly to the Capsule of the kidney and to Bowman's capsule, we will, in order to avoid needless repetition, distinguish between them by employing a capital letter whenever we refer to the former.

4. With the ureter ligatured. This we term an "active obstructed" kidney.

We soon found that the different parts of the renal tubule, and more especially of Bowman's capsule and the glomerulus, varied considerably in size in different animals, so that it is necessary in making comparisons to use only, in the first instance, opposite kidneys in the same animal. Hence, our series of experiments comprises each possible combination in the above-named types of experiments.

All our experiments were performed upon cats anæsthetised with a mixture of chloroform and ether.

In all experiments, the kidney was removed and fixed in the following way. It was first carefully freed from subperitoneal fat, and a ligature then tied tightly around the pedicle close to the hilum. A second ligature was next tied around the pedicle a little nearer the aorta, and the pedicle divided between the ligatures. The object of ligaturing the pedicle was to keep the urine within the tubules, and as far as possible in the position it occupied at the instant of ligature. The kidney was dropped intact into a beaker of 20-per-cent. formalin made up with 0·9 per cent. NaCl. The beaker and solution had previously been weighed, and it was now weighed a second time, giving the weight of the kidney. At the end of an hour, the kidney was sliced into thin sections, fixation in formalin completed, and the pieces imbedded and sections prepared. The following measurements were then taken:—

1. An equatorial diameter of the capsule at right angles to the polar diameter.

2. The polar diameter, *i.e.* one passing through the point of entrance of the blood-vessels.

3. The greatest distance between the glomerulus and the capsule if the two were not in contact.

4. The maximal diameter of a typical proximal convoluted tubule.

5. The diameter of its lumen.

6 and 7. Similar measurements of a typical section of the distal convoluted tubule.

The glomeruli measured were taken at random, care being exercised only to measure those in which the section passed centrally. This was generally fairly easy to attain by taking those which showed the point of entrance of the blood-vessel into the glomerulus. From these measurements, calculations were made of the approximate volumes of the capsule and the glomerulus respectively. To obtain these, we regarded the capsule as equal in volume

to a sphere whose diameter was the mean of the two diameters of the capsule. The figures representing volumes given in this paper were obtained by cubing the mean radius of the capsule expressed in microns, and dividing it by 1000. Hence, to convert the figures into cubic millimetres, they must be multiplied by $4{\cdot}2 \times 10^{-6}$. The glomerulus was also compared to a sphere, whose diameter was the diameter of the capsule minus the maximum space between the glomerular surface and the capsular surface. The difference between the two volumes thus ascertained gives us an approximate estimate of the volume of the fluid contained within the capsule.

In measuring the tubules a section of a proximal convoluted tubule lying near to the glomerulus was selected, and that section of the distal convoluted tubule which lies close to the point of entrance of the vessels into the glomerulus. Hence the proximal tubule probably belonged to the glomerulus measured, and the distal tubule certainly did so belong.

I. *Comparison between a Resting and an Active Kidney.*

A. *The Glomerulus and Capsule.*—There are always marked differences between a resting and an active glomerulus. A resting glomerulus appears to be made up of a dense tissue closely packed with nuclei (fig. 1). The glomerular surface always lies in contact with the capsule wall, and the whole structure is usually irregularly quadrangular in outline. After activity the glomerulus stands away clearly from the capsule. The outline of the glomerulus is lobular, and in structure it is much looser than the resting glomerulus (fig. 2). It also appears to be filled with large vacuole-like spaces approximately circular in section. The nuclei are well separated. As a rule the number of blood corpuscles contained in the glomerular vessels is quite small, far fewer than in the resting glomerulus. This we think may be due to the expulsion of the blood from the capillary loops after excision of the kidney, or to *post-mortem* laking of the corpuscles. The latter may be produced by the diffusion of water from the capsule through the walls of the capillary loops after the epithelial cells have died, and before the fixative has had time to act upon them. This would account for the very characteristic vacuolated appearance of the glomeruli already alluded to.

We were never able to keep the blood in a kidney that was excised at the height of activity. At the instant of excision such a kidney is hard and tense, and instantly becomes soft when the first ligature is tied round the pedicle. This is even the case though the vein be first ligatured, and though the kidney may have been separated from its surrounding tissues before the diuretic was administered in order to give ample time for closure of the

many small vessels passing through the Capsule. Even then there is a distinct escape of blood through the Capsule, and the cortex rapidly pales in colour as the tension falls. The greater the tension at the instant of ligature, the greater is this paling of the cortex, and the sections of such kidneys may show but traces of blood in any of the capillaries, and but little in the veins.

The change in the shape of Bowman's capsule when the kidney becomes active is very distinctive. It becomes circular or elliptic in section, and there is always fluid between the glomerulus and the capsule wall. In many instances we have noted one other highly suggestive appearance. This is that the first portion of the proximal tubule has, in cases in which a free diuresis was established, been distended so as to appear almost a part of the capsule wall. An instance of this is illustrated in fig. 3. It is a very clear indication that the capsule and the first part of the convoluted tubule have been subjected to a high internal pressure. There are further indications, moreover, that the capsule has been distended to a size much larger than it appears in the section after fixation. The action of a high intracapsular pressure also adequately explains the change of shape from irregularly quadrangular to spheroidal or ellipsoidal.

B. *The Tubules.*—The contrast between the tubules at rest and after they have been in activity is just as striking, and in some particulars has already been described by several observers. In this paper we deal entirely with changes in the total diameter and in the lumen of the tubules, and, moreover, restrict our attention for the most part to the two convoluted tubules.

The magnitude of these several changes is brought out by the following measurements taken from Experiment 10. The measurements are in microns, and each is the mean of 10 measurements:—

Expt. 10.—R. kidney resting. L. kidney free.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter ..... | 108·4 | 144·0 |
| Polar diameter ..... | 78·4 | 103·6 |
| Space ..... | 3·0 | 23·8 |
| Hence | | |
| Mean diameter capsule ..... | 93·4 | 123·8 |
| „ „ glomerulus ..... | 90·4 | 100·0 |
| Approximate volume capsule ... | 102 | 237 |
| „ „ glomerulus | 92 | 125 |
| „ „ fluid..... | 10 | 112 |

| | | | |
|---|---|---|---|
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 41·4 | 41·4 |
| | Lumen ..... | 0·0 | 17·6 |
| Distal. | External diameter ... | 21·2 | 32·4 |
| | Lumen ..... | 7·2 | 20·6 |

| | grm. |
|---|---|
| Weight of R. kidney ..... | 10·9 |
| „ L. kidney ..... | 16·2 |

These figures show most clearly how extensive a change in size of the different parts of the renal tubule occurs when it is thrown into activity. Thus the capacity of the capsule is more than doubled (to 232 per cent.), chiefly because of the very large accumulation of fluid which has been secreted. The glomerulus is, however, increased to 136 per cent. of the volume of the glomerulus at rest. The differences are in reality still more marked, for a glomerulus actually at rest has no space between the glomerulus and the capsule wall, whereas in the right kidney of this animal no less than 7 of the 10 capsules measured contained fluid, though but small in amount.

We may conclude, then, that both Bowman's capsule and the glomerulus are distensible structures, and, further, that during activity the glomerulus does not remain in contact with the capsule wall, all of which strongly opposes the filtration theory of glomerular activity. These two conclusions are confirmed by every experiment we have performed.

When we turn to the measurements of the tubules the changes are equally striking. The external diameter of the proximal tubule is usually unaltered, but, whereas the resting tubule has no lumen, the tubule after action has a large lumen (43 per cent. of the total diameter). With the distal convoluted tubule the case is somewhat different. The total diameter is markedly increased (to 153 per cent.). The lumen of the resting tubule is 34 per cent., but that of the active tubule 64 per cent. of the total diameter of the tubule. Also, the lumen of the active tubule is 2·86 times greater than that of the resting. Apparently, then, the basement membrane of the proximal convoluted tubule is practically inextensible with the forces at play in this instance, whereas that of the distal convoluted tubule is extensible. In both tubules the cells are distinctly flattened against the basement membrane as a result of activity.

### II. *Comparison between a Resting and a Decapsulated Kidney.*

The measurements obtained in an experiment of this character (Experiment 11) were as follows:—

Expt. 11.—R. kidney, resting. L. kidney, decapsulated and secreting freely.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter | 100·4 | 112·0 |
| Polar diameter | 73·6 | 95·2 |
| Space | 3·0 | 14·6 |
| Hence | | |
| Mean diameter capsule | 87·0 | 103·6 |
| „ „ glomerulus | 84·0 | 89·0 |
| Approximate volume capsule | 82 | 139 |
| „ „ glomerulus | 74 | 88 |
| „ „ fluid | 8 | 51 |
| Convoluted tubules— | | |
| Proximal. External diameter | 46·0 | 42·0 |
| Lumen | 1·4 | 19·4 |
| Distal. External diameter | 24·0 | 28·0 |
| Lumen | 10·8 | 17·6 |

| | grm. |
|---|---|
| Weight of R. kidney | 8·4 |
| „ L. kidney | 10·6 |

In this experiment the changes are entirely in the same direction as in the preceding, and the magnitude of the various changes is also approximately the same. If anything, the free kidney in the preceding experiment showed rather greater changes in comparison to the resting than did the decapsulated kidney of this experiment. The difference is, however, accounted for by the fact that the diuresis in Experiment 10 was greater than in Experiment 11.

The increase in volume of the capsule is to 170 per cent., of the glomerulus to 119 per cent. One notable difference is that in this experiment the external diameter of the proximal convoluted tubule was less after diuresis than when at rest.

III. *Comparison of a Free Kidney with a Free Decapsulated Kidney.*

Expt. 3.—R. kidney free. L. kidney free and decapsulated.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter | 135·2 | 142·4 |
| Polar diameter | 100·8 | 112·0 |
| Space | 15·2 | 20·6 |

Hence

| | | | |
|---|---|---|---|
| Mean diameter capsule ..... | | 118·0 | 127·1 |
| „ „ glomerulus ..... | | 100·8 | 106·5 |
| Approximate volume capsule..... | | 205 | 257 |
| „ „ glomerulus | | 128 | 151 |
| „ „ fluid..... | | 77 | 106 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 44·8 | 48·0 |
| | Lumen..... | 13·0 | 19·8 |
| Distal. | External diameter ... | 33·2 | 39·2 |
| | Lumen..... | 24·8 | 29·2 |

| | grm. |
|---|---|
| Weight of R. kidney..... | 20·6 |
| „ L. kidney..... | 19·1 |

The two kidneys show the general changes of a diuresis in a well-marked manner. The experiment further shows that the effect of decapsulation is to cause a relatively greater expansion of both capsule and glomerulus. Also, the capsule is not so well emptied as in the normally active kidney. The difference in the dilatation of the convoluted tubules is again in favour of the decapsulated kidney. This is particularly seen with regard to the lumen of the proximal convoluted tubule. Whereas the ratio of the external diameter of the first convoluted tubule of the decapsulated kidney to that of the free kidney is 1 to 1·07, the ratio of the lumina is 1 to 1·53.

Hence we may conclude that decapsulation results in an increased distension of all the cortical parts of the kidney tubule when it is thrown into activity.

In the next group of experiments one of the kidneys was obstructed. The group comprises three comparisons.

IV. *Comparison of a Resting Kidney with an Obstructed Kidney.*

Expt. 12.—R. kidney resting. L. kidney obstructed.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter ..... | 98·4 | 130·4 |
| Polar diameter ..... | 76·0 | 111·2 |
| Space ..... | 1·2 | 24·8 |

Hence

| | | |
|---|---|---|
| Mean diameter capsule ..... | 87·2 | 120·8 |
| „ „ glomerulus ..... | 86·0 | 96·0 |
| Approximate volume capsule..... | 83 | 220 |
| „ „ glomerulus | 80 | 111 |
| „ „ fluid..... | 3 | 109 |

Convoluted tubules—

| | | | |
|---|---|---|---|
| Proximal. | External diameter ... | 44·0 | 42·8 |
| | Lumen..... | 0·0 | 19·4 |
| Distal. | External diameter ... | 25·4 | 31·8 |
| | Lumen..... | 11·0 | 21·8 |

| | grm. |
|---|---|
| Weight of R. kidney ..... | 7·7 |
| „ L. kidney ..... | 10·9 |

The general changes are in the same direction as before. Perhaps the most marked difference between this and the previous kidneys examined is the large volume of fluid contained within the capsule, and the relatively small size of the glomerulus. Again, we note that there is no change in the external diameter of the proximal convoluted tubule, whereas the distal is extended to 125 per cent. of its resting diameter. As illustrated by the lumina, a very considerable volume of urine is collected within the tubules, particularly in the distal tubule.

V. *Comparison of a Free Kidney with an Obstructed Kidney.*

Expt. 6.—L. kidney free. R. kidney obstructed.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter ..... | 99·6 | 110·8 |
| Polar diameter ..... | 77·2 | 100·0 |
| Space ..... | 6·2 | 16·0 |

Hence

| | | |
|---|---|---|
| Mean diameter capsule ..... | 88·4 | 105·4 |
| „ „ glomerulus ..... | 82·2 | 89·4 |
| Approximate volume capsule..... | 86 | 146 |
| „ „ glomerulus | 69 | 89 |
| „ „ fluid ..... | 17 | 57 |

| | | | |
|---|---|---|---|
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 39·2 | 39·4 |
| | Lumen ..... | 6·6 | 14·0 |
| Distal. | External diameter ... | 22·8 | 28·2 |
| | Lumen ..... | 12·6 | 18·4 |

| | grm. |
|---|---|
| Weight of L. kidney ..... | 13·5 |
| „ R. kidney ..... | 15·5 |

This experiment shows quite clearly the great effect of obstruction upon the distension of the capsule and accumulation of fluid within the capsule. Obstruction also causes a distinct further dilatation of the distal convoluted tubule, and an increase in the lumina of both parts of the tubule.

VI. *Comparison of a Free Decapsulated Kidney with an Obstructed Kidney.*

Expt. 7.—R. kidney decapsulated. L. kidney obstructed.

| | | R. μ. | L. μ. |
|---|---|---|---|
| Glomeruli and capsules— | | | |
| Equatorial diameter ..... | | 121·2 | 130·4 |
| Polar diameter ..... | | 102·0 | 111·2 |
| Space ..... | | 9·8 | 20·0 |
| Hence | | | |
| Mean diameter capsule ..... | | 111·6 | 120·8 |
| „ „ glomerulus ..... | | 101·8 | 100·8 |
| Approximate volume capsule ... | | 174 | 220 |
| „ „ glomerulus | | 132 | 128 |
| „ „ fluid ..... | | 42 | 92 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 42·0 | 41·4 |
| | Lumen ..... | 15·4 | 17·6 |
| Distal. | External diameter ... | 28·6 | 31·0 |
| | Lumen ..... | 20·4 | 21·2 |

| | grm. |
|---|---|
| Weight of R. kidney ..... | 10·7 |
| „ L. kidney ..... | 11·0 |

The results of the measurements in this experiment show that obstruction of the ureter results in an increased expansion of the capsule of the obstructed, as compared to that of the free active kidney; this is entirely due to a greater accumulation of fluid within it. The convoluted tubules

show corresponding differences. The effect as before is mainly felt in the distal tubule, which shows a somewhat greater expansion. The lumina in the proximal tubules are greater in the obstructed kidney than in the free kidney. In this experiment the blood-pressure was rather low, but the diuresis good.

In all these obstructed kidneys the effect upon the medulla is very marked. Not only is the pelvis of the kidney greatly distended, but the pyramid is driven back towards the cortex, and appears very much shrunken. We have often seen it so contracted as to appear only about a quarter or less of its normal size. In the sections the collecting tubules are flattened and empty, the loops of Henle, however, contain fluid, and often appear to be about the same size as in the normal active kidney. The appearance of the pyramids is so characteristic that one can at once decide whether or no the ureter of that kidney had been obstructed in the experiment.

The last group of experiments comprises a comparison of various kidneys with a kidney which was both obstructed and decapsulated.

VII. *Comparison of a Resting Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 13.—R. kidney resting. L. kidney decapsulated and obstructed.

| | | R. μ. | L. μ. |
|---|---|---|---|
| Glomeruli and capsules— | | | |
| | Equatorial diameter ..... | 110·4 | 128·0 |
| | Polar diameter ..... | 79·6 | 110·8 |
| | Space ..... ..... | 3·4 | 21·2 |
| Hence | | | |
| | Mean diameter capsule ..... | 95·0 | 119·4 |
| | „ „ glomerulus..... | 91·6 | 98·2 |
| | Approximate volume capsule..... | 107 | 213 |
| | „ „ glomerulus | 96 | 118 |
| | „ „ fluid..... | 11 | 95 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 46·0 | 49·6 |
| | Lumen..... | 0·0 | 26·4 |
| Distal. | External diameter ... | 21·8 | 34·4 |
| | Lumen..... | 10·6 | 24·4 |

| | grm. |
|---|---|
| Weight of R. kidney ..... | 14·5 |
| „ L. kidney ..... | 19·3 |

An examination of the figures brings out an enormous increase in the size of the capsules, due chiefly to the increase in the amount of the fluid contained. The effect upon the convoluted tubules is again most marked. Otherwise the figures require no further comment.

The right kidney was not completely at rest, as was indicated by the microscopic appearance of the glomeruli. In every instance there was fluid between the glomerulus and the capsule.

VIII. *Comparison of a Free Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 1.—R. kidney free. L. kidney decapsulated and obstructed.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter ..... | 135·6 | 143·6 |
| Polar diameter ..... | 106·8 | 125·6 |
| Space ..... | 23·8 | 31·6 |
| Hence | | |
| Mean diameter capsule ..... | 121·2 | 134·6 |
| „ „ glomerulus..... | 97·4 | 103·0 |
| Approximate volume capsule..... | 223 | 305 |
| „ „ glomerulus | 116 | 137 |
| „ „ fluid ..... | 107 | 168 |
| Convoluted tubules— | | |
| Proximal. External diameter ... | 48·2 | 51·4 |
| Lumen..... | 13·2 | 24·0 |
| Distal. External diameter ... | 38·6 | 39·8 |
| Lumen..... | 29·0 | 30·8 |

The general result of the experiment shows that the glomeruli and convoluted tubules are more distended in the decapsulated and obstructed kidney than in the free kidney. In this instance the volume of the capsules became enormous, with only a slight increase in the volume of the glomeruli. We would emphasise the very great size of the lumen of the proximal convoluted tubule.

Expt. 4.—R. kidney free. L. kidney decapsulated and obstructed.

| | R. μ. | L. μ. |
|---|---|---|
| Glomeruli and capsules— | | |
| Equatorial diameter ..... | 137·6 | 152·4 |
| Polar diameter ..... | 112·4 | 122·4 |
| Space ..... | 10·4 | 22·0 |

Hence

| | | | |
|---|---|---|---|
| Mean diameter capsule ..... | | 125·0 | 137·4 |
| „ „ glomerulus..... | | 114·6 | 115·4 |
| Approximate volume capsule ... | | 244 | 324 |
| „ „ glomerulus | | 188 | 192 |
| „ „ fluid..... | | 56 | 132 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 45·6 | 43·4 |
| | Lumen ..... | 15·2 | 22·8 |
| Distal. | External diameter ... | 27·8 | 29·4 |
| | Lumen ..... | 15·8 | 21·2 |

| | grm. |
|---|---|
| Weight of R. kidney..... | 16·5 |
| „ L. kidney ..... | 18·7 |

The results obtained in this experiment in every way confirm those shown in the previous experiment.

IX. *Comparison of a Decapsulated Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 2.—L. kidney decapsulated. R. kidney decapsulated and obstructed.

| | | L. μ. | R. μ. |
|---|---|---|---|
| Glomeruli and capsules— | | | |
| Equatorial diameter ..... | | 142·4 | 161·2 |
| Polar diameter ..... | | 122·4 | 129·2 |
| Space ..... | | 6·2 | 12·4 |

Hence

| | | | |
|---|---|---|---|
| Mean diameter capsule ..... | | 132·4 | 145·2 |
| „ „ glomerulus..... | | 126·2 | 132·8 |
| Approximate volume capsule ... | | 290 | 383 |
| „ „ glomerulus | | 251 | 293 |
| „ „ fluid..... | | 39 | 90 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 47·4 | 47·2 |
| | Lumen ..... | 12·4 | 19·2 |
| Distal. | External diameter ... | 30·4 | 36·4 |
| | Lumen ..... | 18·8 | 24·2 |

| | grm. |
|---|---|
| Weight of L. kidney ..... | 13·6 |
| „ R. kidney..... | 16·6 |

The results of the experiment are again very decisive, a notable point being the large volume of the glomeruli in both kidneys. We would again point out that the main effect upon the convoluted tubules is seen in the distal tubules.

Expt. 5.—R. kidney decapsulated. L. kidney decapsulated and obstructed.

| | | R. μ. | L. μ. |
|---|---|---|---|
| Glomeruli and capsules— | | | |
| | Equatorial diameter ..... | 144·8 | 151·6 |
| | Polar diameter ..... | 111·6 | 136·8 |
| | Space ..... | 24·0 | 37·6 |
| Hence | | | |
| | Mean diameter capsule ..... | 128·2 | 144·2 |
| | „ „ glomerulus..... | 104·2 | 106·6 |
| | Approximate volume capsule ... | 263 | 375 |
| | „ „ glomerulus | 141 | 151 |
| | „ „ fluid ..... | 122 | 224 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 45·6 | 51·0 |
| | Lumen..... | 20·8 | 25·2 |
| Distal. | External diameter ... | 34·0 | 42·0 |
| | Lumen ..... | 23·0 | 30·6 |

| | grm. |
|---|---|
| Weight of R. kidney..... | 17·6 |
| „ L. kidney ..... | 18·0 |

The figures are in agreement with those of the preceding experiment, with the exception that the volume of the glomeruli in this instance is small. In Experiment 2 the blood-pressure was low (83 mm. Hg) and the diuresis moderate, while in Experiment 5 the blood-pressure was high (130 mm. Hg) and the flow of urine rapid.

X. *Comparison of an Obstructed Kidney with a Decapsulated and Obstructed Kidney.*

Expt. 8.—L. kidney obstructed. R. kidney decapsulated and obstructed.

| | | L. μ. | R. μ. |
|---|---|---|---|
| Glomeruli and capsules— | | | |
| | Equatorial diameter ..... | 109·2 | 108·0 |
| | Polar diameter ..... | 95·6 | 95·2 |
| | Space ..... | 11·8 | 9·2 |

Hence

| | | | |
|---|---|---|---|
| Mean diameter capsule ..... | | 102·4 | 101·6 |
| „ „ glomerulus ..... | | 90·6 | 92·4 |
| Approximate volume capsule ... | | 134 | 131 |
| „ „ glomerulus | | 93 | 99 |
| „ „ fluid..... | | 41 | 32 |
| Convoluted tubules— | | | |
| Proximal. | External diameter ... | 40·4 | 41·4 |
| | Lumen..... | 17·8 | 18·2 |
| Distal. | External diameter ... | 25·4 | 29·6 |
| | Lumen..... | 17·2 | 21·0 |

| | grm. |
|---|---|
| Weight of R. kidney ..... | 15·8 |
| „ L. kidney ..... | 15·8 |

In this experiment the blood-pressure was low and the flow of urine small, and with it again the volume of fluid in the capsule is small. In general, it confirms the result of the preceding experiments. Decapsulation combined with obstruction produces a greater distension of the tubules than obstruction alone. With a more abundant diuresis than occurred in this experiment a similar result is found in the capsules and glomeruli.

In the following tables we collect the results obtained in our thirteen experiments. In the first we give the means of the approximate volumes of Bowman's capsule, glomerulus and fluid, and in the second the ratios of these to the similar structures in the resting kidney.

We would not lay much stress upon comparisons between these figures, except when the differences are very marked. There are so many varying factors upon which the actual magnitudes of the measurements depend that to do so would lead to erroneous conclusions. Thus, the results vary with the blood-pressure, with the degree of diuresis established, the duration of the diuresis, and especially with the degree of extensibility of the kidney Capsule, and of the general renal tissues, both of which we know to vary greatly in different animals. Table II, however, shows very decisively the enormous changes in size of the glomerulus and capsule caused by active secretion of water, and more especially in the very great accumulation of water within the capsule during activity. All these results are of the highest importance in disproving the possibility of filtration at the glomerular surface.

Table I.

| | Volume Bowman's capsule. | Volume glomerulus. | Volume fluid. | No. of experiments. |
|---|---|---|---|---|
| Resting | 94 | 85 | 9 | 4 |
| Active free | 227 | 137 | 90 | 4 |
| „ decapsulated | 229 | 162 | 67 | 5 |
| „ obstructed | 196 | 136 | 60 | 3 |
| „ decapsulated and obstructed | 277 | 157 | 120 | 6 |

Table II.—Ratios.

| | Bowman's capsule. | Glomerulus. | Fluid. |
|---|---|---|---|
| Resting | 1·00 | 1·00 | 1·00 |
| Active free | 2·42 | 1·61 | 10·00 |
| „ decapsulated | 2·44 | 1·91 | 7·44 |
| „ obstructed | 2·09 | 1·60 | 6·67 |
| „ decapsulated and obstructed | 2·95 | 1·85 | 13·34 |

In Tables III and IV we give similar figures for the convoluted tubules.

Table III.

| | Proximal. | | Distal. | |
|---|---|---|---|---|
| | External diameter. | Lumen. | External diameter. | Lumen. |
| Resting | 44·4 | 0·4 | 23·4 | 9·9 |
| Active free | 45·0 | 14·8 | 33·0 | 22·6 |
| „ decapsulated | 46·0 | 17·3 | 33·2 | 22·2 |
| „ obstructed | 42·0 | 19·0 | 29·3 | 20·3 |
| „ decapsulated and obstructed | 47·3 | 22·6 | 35·3 | 25·4 |

Table IV.—Ratios.

| | Proximal. | | Distal. | |
|---|---|---|---|---|
| | External diameter. | Lumen. | External diameter. | Lumen. |
| Resting | 1·00 | 1·00 | 1·00 | 1·00 |
| Active free | 1·01 | 37·00 | 1·41 | 2·28 |
| „ decapsulated | 1·04 | 43·25 | 1·42 | 2·24 |
| „ obstructed | 0·95 | 47·50 | 1·25 | 2·05 |
| „ decapsulated and obstructed | 1·07 | 56·50 | 1·51 | 2·57 |

These two tables bring out the following points:—

(1) The external diameter of the proximal convoluted tubule does not change on activity;

(2) A large lumen is developed in this tubule during diuresis. It varies with the degree of diuresis, and is markedly increased by obstruction of the ureter. Taking the average of all our observations it amounts to nearly 40 per cent. of the total diameter of the tubule;

(3) The distal convoluted tubule is expanded considerably (from 140 to 150 per cent. of its mean at rest); and

(4) The lumen, of considerable size (42·3 per cent. of the total diameter) even in a resting kidney, is more than doubled, and becomes 69·2 per cent. of the total diameter.

We may conclude, then, that the first convoluted tubule, *i.e.* that portion which is subjected to the highest internal pressure, is relatively inextensible transversely. The second convoluted tubule, on the other hand, is transversely extensible. From a further examination of our sections, we judge that the proximal convoluted tubules do indicate an extension in the longitudinal direction, but our present methods do not allow us to state this decisively.* All the results indicate that an internal pressure has existed during diuresis.

*Conclusions.*

Measurements of the diameters of the various portions of the renal tubule in the cat, when at rest and after diuresis under various conditions, show that Bowman's capsule, the glomerulus, and the second convoluted tubule are extensible structures, and are expanded during diuresis. The glomerulus leaves the capsule wall, a considerable accumulation of secretion being found between them. The lumina of all parts of the tubule become greatly enlarged.

All the appearances found are explained as resulting from the action of a high pressure in the fluid secreted by the glomerular epithelium, and are all in accordance with the propulsor theory of the action of the glomerulus.

* If we may make the assumption that the volume of the cells of the convoluted tubule does not alter during diuresis, then the magnitude of the surface areas of the cells in a transverse section of the tubule gives us an indication of any change in length. If, for this purpose, we examine the results of Experiments 10, 11, 12, and 13, where we have direct comparisons of active with resting kidneys, we find that in all instances the proximal convoluted tubules are markedly stretched longitudinally. In Experiments 10 and 13 there is considerable shortening of the distal convoluted tubules, and in Experiments 11 and 12 slight shortening. In Experiments 10 and 13 the blood-pressure was high and the diuresis good. In Experiments 11 and 12 the blood-pressure was lower and the diuresis only moderate. Hence it would appear that, with a high internal pressure, this portion of the tubule is shortened, *i.e.* tends towards the spherical shape.

DESCRIPTION OF PLATE.

Fig. 1.—Microphotograph of Cortex of Dog's Kidney at Rest. ×500.

Fig. 2.—Microphotograph of Cortex of Opposite Kidney after Activity. ×500.

Fig. 3.—Cat's Kidney. Drawing of glomerulus and tubules after activity, showing dilatation of neck of tubule. ×500.

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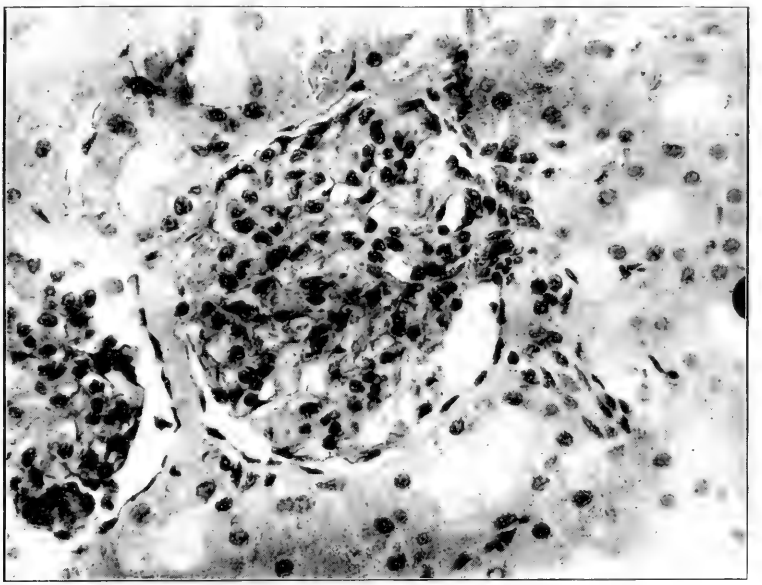

FIG. 1.

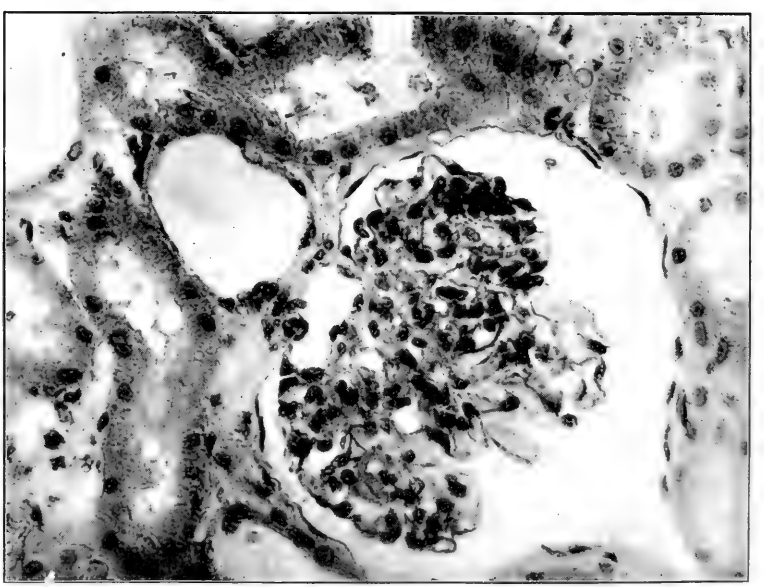

FIG. 2.

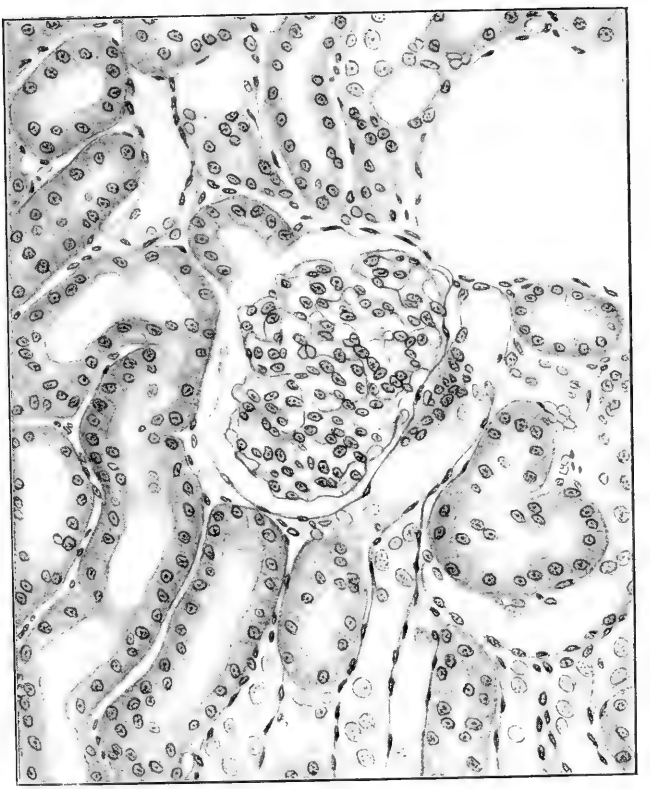

FIG. 3.

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# FURTHER OBSERVATIONS ON THE DIFFERENTIAL ACTION OF ADRENALIN

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Received for publication March 17, 1917

In the course of a series of experiments performed by one of us and reported in this Journal (1), it was found that the fall in general blood pressure, which is caused by the intravenous injection of small doses of adrenalin, is not brought about by dilatation in the vessels of all parts of the body alike. In an animal in which the arteries to the abdominal organs have been clamped, injections of a standard small dose of adrenalin caused much the same fall as had previously occurred in the intact circulation. On the other hand, when the arteries to the limbs were occluded, those to the splanchnic area being intact, the reaction to the standard dose was changed from a pure fall to a rise of blood pressure, as registered from the carotid artery. In that research the only distinction drawn was the broad one as between the reactions of the "peripheral" circulation on the one hand, which included the vessels of bone, muscle, and skin, and that of the "splanchnic" circulation on the other, which, as well as comprising the vessels of the abdominal and thoracic viscera, necessarily included those of the muscles of the thorax and back. The present research was undertaken with a view to following out the subject of the differential action of adrenalin somewhat more in detail, and in the hope of arriving at some conclusion as to the mechanism involved in the vascular adjustment caused by it.

Oncometric experiments were carried out on intestine, spleen, and kidney. While our research was in progress the appearance of the paper by Hoskins, Gunning and Berry (2) made further investigation of the reactions in skin and muscle unnecessary.

In every case simultaneous records were taken of the reactions of at least two organs in response to adrenalin, since we considered it of importance to determine whether the same range of dose which caused constriction in any one abdominal organ also caused constriction in all the others, and whether the amount giving rise to dilatation in one was

necessarily the same as that which caused another to dilate. Since the reaction of any organ to a given dose may vary not only among different individuals, but also in the same individual during the period of an experiment, it is necessary to record the changes occurring in the same animal, at the same time.

The animals used were dogs and cats. In two of the experiments on the latter we injected urethane subcutaneously; in all the others the anesthetic was ether. Blood pressure was registered from the right carotid artery. Injections of adrenalin were made with a graduated syringe, the needle of which was thrust through the wall of a piece of rubber tubing fitted to a cannula, which was inserted low in the left jugular vein. The adrenalin solution used was that manufactured by Parke, Davis and Company, 1 : 1,000, diluted with distilled water to the required strength immediately before use. In each experiment the first injections were made of a solution 1 : 100,000; when large doses were required, as was often the case in working with dogs, in preference to injecting large quantities of distilled water into the animal's circulation, we substituted for the more dilute solution one of a strength of 1 : 10,000. The duration of each injection was signaled on the record below the time marker. No special precautions were taken as to absolute uniformity in the rate of injection, but it was kept fairly constant, and was in all cases slow, as shown by the records.

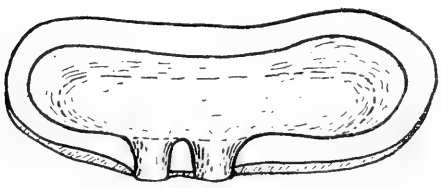

Fig. 1. Gutta percha oncometer for spleen

In some experiments we left the vagi intact; in the majority they were cut. We were unable to observe, however, any specific effect of these on the reaction of any organ to adrenalin except the familiar one of cardiac inhibition caused by large doses, with the consequent great rise in blood pressure.

The oncometers which we used for kidney and for intestine were gutta percha ones of the ordinary type, fitted with glass lids. The early experiments on the spleen were done with the same oncometers; later we had a series of special ones made. These were modelled after the shape of the spleen (see fig. 1) and were provided with two lips for stalks, separated by about 0.6 cm. in the smaller and 1.5 cm. in the larger.

As recorders in the first few experiments we used Marey's drums;

later we substituted for these bellows recorders, which have the advantage of recording volume changes without introducing alterations of pressure within the system itself. In several experiments the recorders were calibrated by injections of known volumes of air.

The pressure inside the system differed little from atmospheric; in practice we raised the pressure until the bellows were about half filled, and were thus adjusted to give maximum variations in either direction.

INTESTINE

A loop of the small intestine, about one-third of its total length, was selected, generally that immediately above the caecum, since the blood vessels there are long and form a convenient stalk. Two pairs of double ligatures were tied about its lower end, about 2 inches apart, the blood vessels supplying the piece between the ligatures tied off, and the piece of intestine removed. A similar operation was performed at the upper end of the required length, and the loop was ready to be placed in the oncometer without the necessity of further dissection, other than simply slitting the mesentery for an inch or two on either side of the stalk. Before putting the loop into the oncometer we washed out its contents with warm saline. This prevents the slow formation of gas which otherwise takes place within the lumen, and which interferes with the records of volume changes due to the circulation alone. The whole operation from the time the abdomen was opened until the intestine was put into the oncometer was not longer than fifteen or twenty minutes. During this time the intestinal loop and the other abdominal contents were kept covered with warm saline pads.

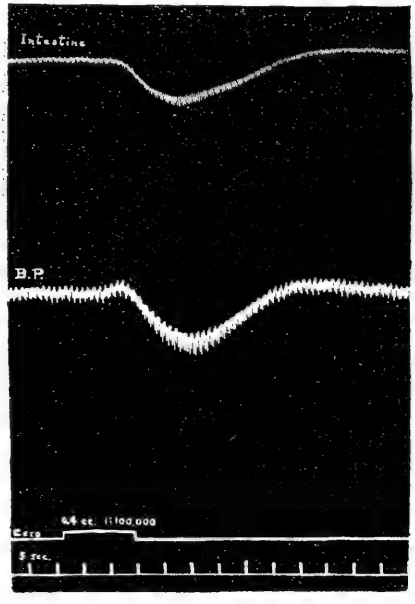


Fig. 2. Constriction of the intestine following injection of a small dose of adrenalin (0.4 cc., 1 : 100,000). Dog 14 (Reduced $\frac{1}{3}$)

In the great majority of cases, the effect of doses of adrenalin, both large and small, was to cause constriction of the intestine. In all of

these experiments small doses caused only constriction (figs. 2 and 6); as the quantity of adrenalin was increased, however, a prolonged and marked dilatation supervened on the preliminary constriction (see figs. 3 and 7). There were two exceptions, in which the least effective dose caused dilatation. These occurred during the early experiments, before we had fully realized the importance of completely removing gas from the lumen, and we consider it probable that this increase in volume of the loop was caused by the relaxation of the muscles of its walls, under the influence of the adrenalin.

The threshold for the constrictor effect was shown, in the six experiments in which it was determined, to vary within fairly wide limits, from 0.014 to 0.07 cc. adrenalin 1 : 100,000 per kilogram body weight, that is, it was reached by doses such as also caused a slight fall in blood pressure. The general resemblance of these two curves, indeed, make it at first glance appear possible that the one effect may be dependent on the other, and that the constriction in the intestine may be nothing more than a decrease of blood supply to its vessels, caused by the lowering of the general blood pressure. Latent periods, duration, and degree of decrease of intestinal volume, also bear some relationship to the same changes in the general blood pressure. Closer inspection of the tables (1 and 2), however, shows clearly that this is not the case. Though the diminution in intestinal volume generally occurs several seconds after the beginning of the fall of blood pressure, this is not alway s the case; for instance in experiments 13, 14, and 20, the records show the intestinal decrease to precede that of the blood pressure by several seconds, and our notes, made during the course of the experiments, corroborate this as actually occurring, and not being due to a possible faulty alignment. The time of the least intestinal volume does not correspond to that of the lowest blood pressure, nor is the duration of the constriction the same as that of blood pressure fall. (See experiments 7 and 13, where it is greater, and experiments 3, 18 and 20, where it is materially less.) Above all, the constriction does not take place only when the dose is such as to cause a fall of blood pressure; constriction of the intestine occurs time and time again when the blood pressure is above and not below its normal level (see figs. 3 and 7).

The dose of adrenalin necessary to cause a dilatation of the intestine to follow on this constriction is as variable as is the threshold dose for the constriction itself. It varies from 0.04 to 0.31 cc. of a solution 1 : 100,000 per kilogram in dogs, and in cats it is about 0.4 cc. The latent period of the dilatation is longer than that of the constriction,

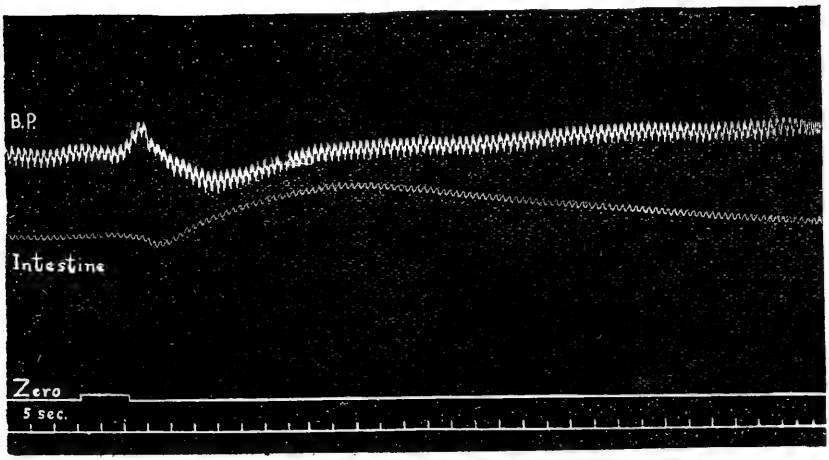


Fig. 3. Preliminary constriction followed by prolonged dilatation of the intestine, caused in the same animal as that of figure 2, by a larger dose (0.2 cc., 1 : 10,000) (Reduced $\frac{1}{3}$)

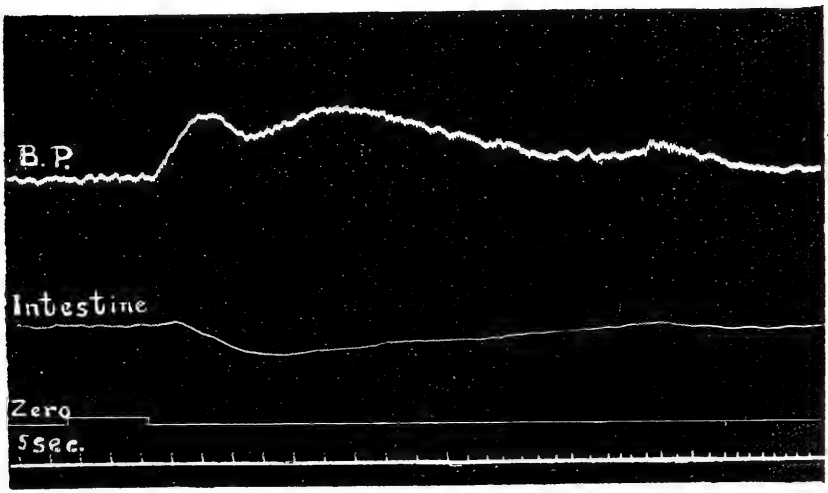


Fig. 4. Reaction of same loop of intestine as that of figures 2 and 3, to a dose of adrenalin of much the same magnitude (0.3 cc., 1 : 10,000) as that of figure 3, after the coeliac and superior mesenteric ganglia had been removed. (Reduced $\frac{1}{3}$)

and the effect is as if the one were superimposed upon the other. As the doses are increased from the threshold dose on, the resulting constriction becomes more and more marked and its duration longer. Once the dose is great enough, however, to cause dilatation, this cuts short the first effect, with the result that the latter is reduced by from one-fourth to two-thirds of its former length. The volume change brought about by dilatation is much larger than that caused by constriction. For instance, in a dog of 25 kilograms the constriction reduced the volume of the system of the intestinal oncometer by 1 cc., while the dilatation increased it by more than 5 cc.

The observations on the resemblance of the curve of constriction to that of the general blood pressure may be applied in much the same way to this case also, for in increasing the doses of adrenalin sufficiently to cause a dilator effect in the intestine, in a few cases we crossed the threshold for pressor effect on the blood pressure. The same arguments, however, which prevented our accepting the explanation of a passive effect in construction, are also valid in this case. The latent periods of intestinal effect are longer than those of blood pressure fall, the time of maximum dilatation never coincides with that of maximum rise of pressure, and its duration is far greater, in many instances two to three times as long (experiments 21, 22, 23, table 2). As before, too, the occurrence of the intestinal effect does not depend on the nature of the blood pressure change; we have several records which, like experiments 3 and 7, show a marked increase in intestinal volume during a fall in general blood pressure.

As a possible explanation for the occurrence of increase in the intestinal volume, after injections of doses of adrenalin above a certain size, it might be suggested that such doses are just sufficient to affect the intrinsic nervous system of the intestine and to bring about relaxation of its walls, thus permitting expansion of the blood vessels within them. To investigate this, we inserted a rubber balloon into a part of the intestine immediately adjacent to that which furnished the loop in the oncometer, injected a small quantity of air, and connected it to a small bellows recorder, which made a tracing below the oncometer record. By this it was found that injections so small as to cause only a slight fall in blood pressure were sufficient to bring about a relaxation of the intestinal wall, and that as the dose was increased no well-marked difference could be observed in the reaction of the intestinal wall to that dose which first gave dilatation in the oncometer, nor to any of the succeeding ones.

In an attempt to decide upon the origin of this dilator effect of adrenalin we severed connection between the loop of intestine involved and the central nervous system, by dissecting and removing the two coeliac ganglia and the superior mesenteric ganglion, or by cutting the splanchnic nerves. In all experiments, five in all, after this operation was performed, the dilatation by adrenalin was entirely done away with, and doses which previously had caused a preliminary constriction followed by a dilatation now gave nothing but a simple constriction of the loop (see fig. 4).

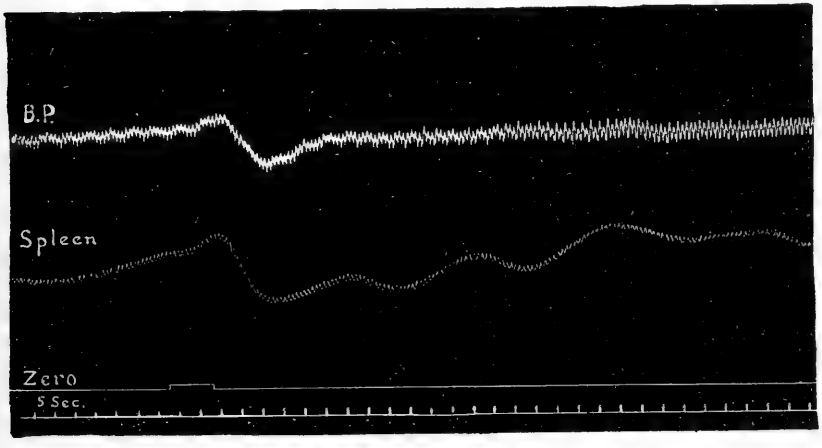


Fig. 5. Constriction in spleen, followed by a series of waves, after injection of a small dose of adrenalin (0.1 cc., 1 : 10,000). Dog 19 (Reduced $\frac{1}{2}$)

## THE SPLEEN

In the dissection of the spleen the gastrosplenic ligament with its numerous fat vessels was ligatured off, bit by bit, and cleared away from the neighborhood of the splenic blood vessels. In the early experiments those of the latter which supply the upper half of the spleen were also tied off. After two or three of these dissections, however, we were so dissatisfied with the appearance of the spleen under these conditions that we adopted a splenic oncometer with two lips. This enabled us to leave all vessels supplying the splenic substance intact, except sometimes that to the extreme tip of the upper end, which bound the organ too closely to allow of its being put into the oncometer. With the exception of this small piece, the spleen remained in

excellent condition, even during the course of an experiment lasting over several hours. During dissection we protected it with saline pads, and we warmed the oncometer to receive it.

Of the dogs, ten in all, which we investigated, seven showed only constriction in the spleen in response to the whole range of doses of adre-

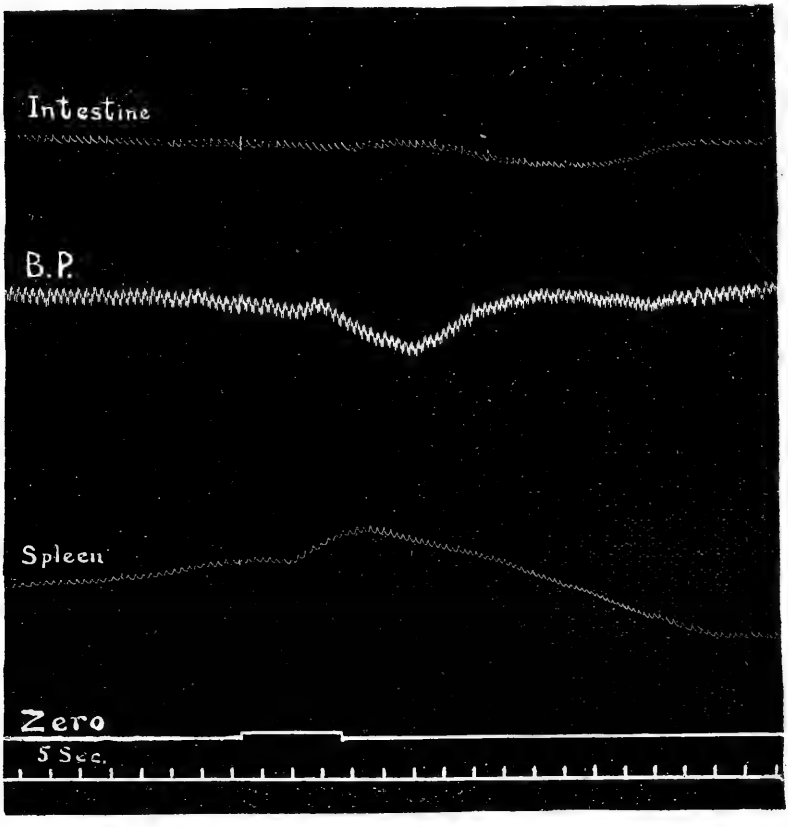


Fig. 6. Dilatation in spleen, and constriction in intestine, after small dose of adrenalin (0.3 cc., 1 : 10,000). Dog 21 (Reduced $\frac{1}{3}$)

nalin employed. This constriction was more marked and more prolonged as the doses were increased in magnitude (see figs. 5 and 7). The three others each gave dilatation at some dose of adrenalin. Two of the three showed as a first effect dilatation with small doses; or, to speak more accurately, small doses of adrenalin set up in these

spleens (which had previously been relatively inactive) a series of waves, of which the first was in the direction of dilatation (see fig. 6). In both these organs the effect of increasing the dose was to increase the constriction in the waves at the expense of the dilatation, until large doses caused only decrease in volume. In the third of these spleens doses of adrenalin also set up series of waves, but its reaction differed

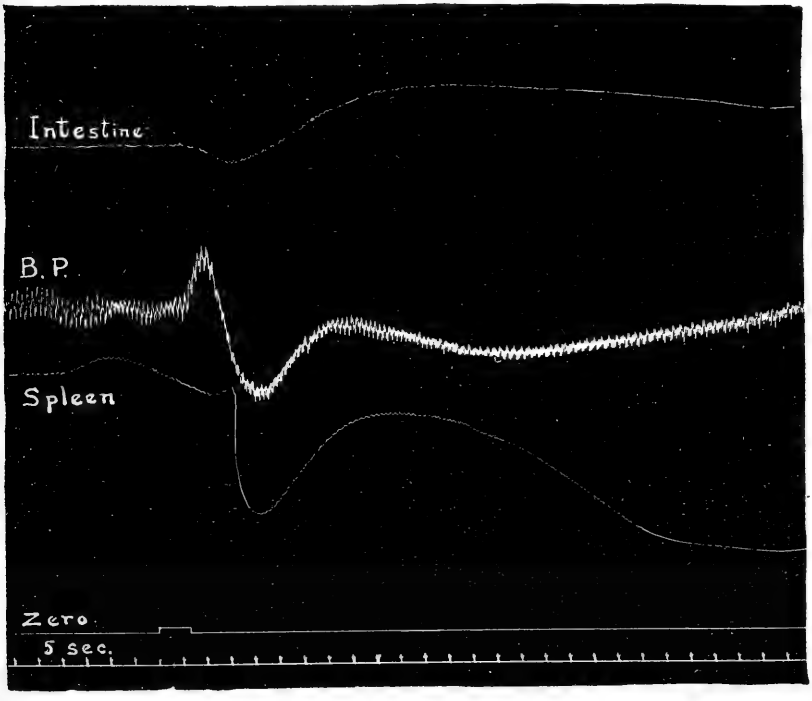


Fig. 7. Effect on same animal as that of figure 6, of a larger dose (1 cc., 1:10,000); slight dilatation followed by marked constriction in spleen, preliminary constriction and marked dilatation in intestine (Reduced $\frac{1}{2}$)

from that of the other two in that, on administration of relatively large doses (0.2 cc., 1 : 100,000 per kilogram) the constriction was followed by dilatation.

### KIDNEY

The upper part of the ureter and the kidney vessels of one side throughout their entire length were dissected out to form a stalk. The mesentery was removed as gently as possible from the surface of the

kidney. A few of the larger veins running from it into the capsule were ligatured. During the dissection the kidney was protected as completely as possible with warm pads.

Four experiments in all were performed, two on cats and two on dogs. In every case injections of adrenalin caused constriction in the kidney (see figs. 8 and 9); with small doses of low concentration this

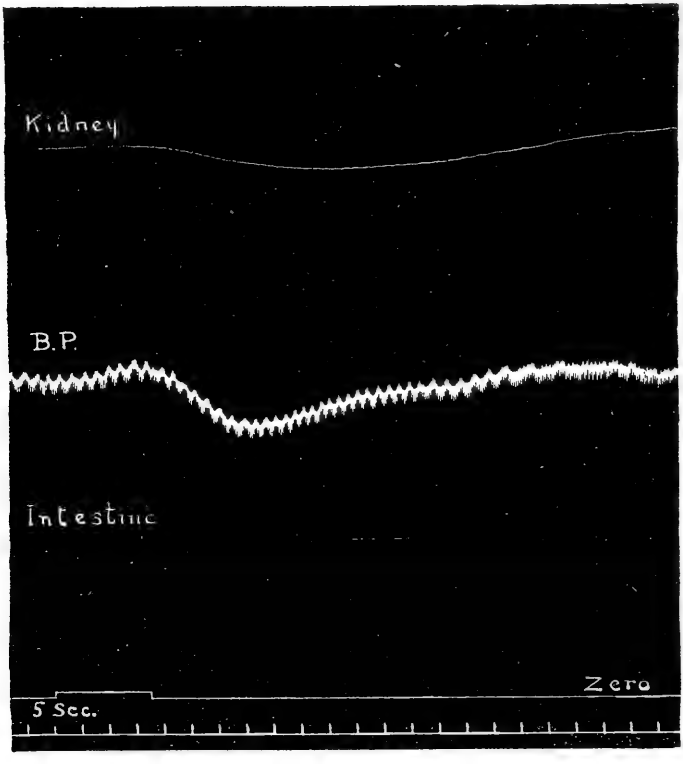


Fig. 8. Constriction of kidney after a dose of adrenalin 0.2 cc., 1 : 100,000 (Reduced $\frac{1}{2}$)

was the only effect (see fig. 8); in two cases the preliminary constriction caused by large doses (e.g., 0.32 cc. 1 : 100,000 per kilogram), such as occasioned a preliminary rise followed by a fall of blood pressure, was followed by a dilatation of the organ. The curve of this dilatation was similar in form to that familiar to us in the reaction of the intestine to large doses of adrenalin. It showed a rise of

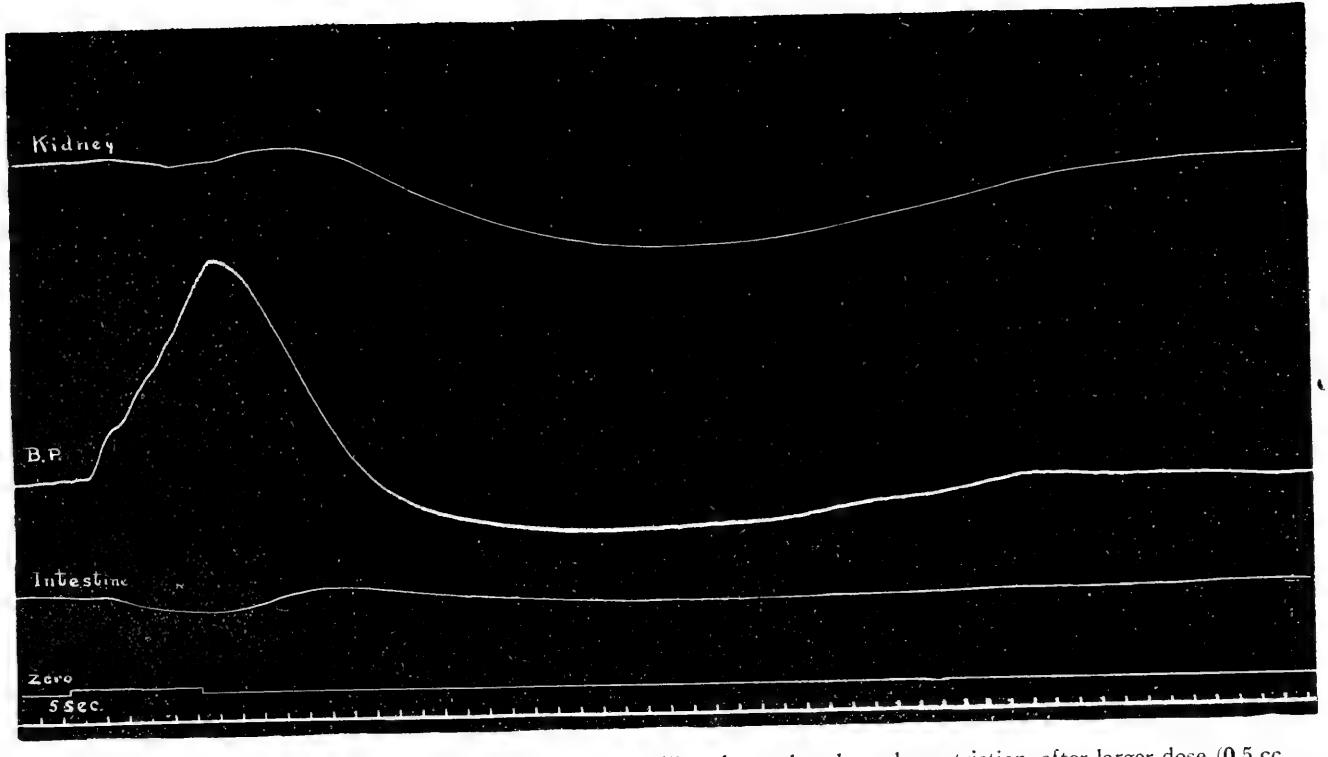


Fig. 9. Reaction of same kidney as in fig. 8, preliminary dilatation and prolonged constriction, after larger dose (0.5 cc., 1 ; 10,000) (Reduced $\frac{1}{2}$).

the lever, which only gradually returned to the base line, not before 170 to 180 seconds had elapsed, and thus continued long after the blood pressure had regained its normal level. The cuase of the occurrence of this dilatation we were not able to determine.

In conclusion we wish to point out that the reactions of the various organs, though they may be of a similar nature, do not necessarily take place at the same time, nor for the same dose. Thus, for instance, in dog 23, table 2, though both kidney and intestine give constriction in response to small doses and dilatation in response to large ones, nevertheless that dose of adrenalin (0.4 cc., 1:100,000 per kilogram) which is enough to cause transition from a dilatation to a constriction in the intestine, still gives rise to nothing but a constriction in the kidney. Numerous other examples may be found by reference to that table.

That the output of adrenalin, which has been shown by the work of the last few years (3), (4), (5), to be so small during normal quiet life as to have no appreciable effect on blood pressure, is augmented during conditions of mental excitement, as well as by the asphyxia attendant on violent exercise, and by sensory stimulation, has been shown in a series of experiments by Cannon and the workers in his laboratory (6). The exact extent of this increase in secretion has not been determined, nor is it known whether it is sufficient to effect a rise rather than a fall in blood pressure. Elliott (7), in working on the secretion from the adrenal glands which is brought about by stimulation of the splanchnic nerves supplying them, has shown that in this case the quantity secreted is within the range of doses which have a depressor effect on the general blood pressure; whether this is also the case during the reflex stimulation of normal life, we are still ignorant. In any case, as shown by our experiments, the first effect of the outpouring of adrenalin during excitement must be to cause a constriction of the intestine and kidney, and generally, though not always, a similar constriction in the spleen. By this means there is brought about a shifting of the blood from these organs to the muscles, which, as Hoskins, Gunning and Berry (2) have shown, are at the same time actively dilated. If, as may prove to be the case, the output of adrenalin increases till the concentration in the arterial blood is of the order of about one-half or more than necessary to bring about a rise in blood pressure, a dilatation of the intestine, and perhaps also of the kidney, must take place, through the agency of some central mechanism, the location of which, and the source of stimulation to which, are as yet unknown.

TABLE 1

*Shortening of the duration of intestinal constriction by the occurrence of dilatation*

| ANIMAL | WEIGHT | AMOUNT OF ADRENALIN INJECTED, CUBIC CENTIMETERS OF 1:100,000 DILUTION PER KILOGRAM OF BODY WEIGHT | LATENT PERIOD | MAGNITUDE OF CONSTRICTION | DURATION OF CONSTRICTION WHEN DILATATION IS ABSENT | DURATION OF CONSTRICTION WHEN DILATATION IS PRESENT | LATENT PERIOD | BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY | DURATION OF BLOOD PRESSURE CHANGE |
|---|---|---|---|---|---|---|---|---|---|
| | *kgms.* | | *seconds* | | *seconds* | *seconds* | *seconds* | | *seconds* |
| Cat. 4..... | 3.15 | 0.06 | | Marked | 160 | | | Fall 136 to 110 | 160 |
| | | 0.16 | | Marked | | 110 | | Fall 138 to 110 | 215 |
| | | | | Small | 97 | | | 175 to 181 to 142 | 97 |
| Cat 7..... | | | | Small | | 13 | | 164 to 218 | 63 |
| | | | 12 | Marked | | 28 | | 170 to 235 to 151 | 240+ |
| | 7 | 0.03 | 11 | Marked | 32 | | | 102 to 90 | 33 |
| Dog 14..... | | 0.14 | 10 | Very marked | | 28 | | 99 to 87 | 23 |
| | | 1.26* | | Marked | | 15 | | 96 to 180 | 105 |
| | 9.5 | 0.05 | 12 | Marked | 33 | | 11 | 160 to 130 | 60 |
| Dog 20..... | | 0.31 | 10 | Marked | | 21 | 11 | 150 to 122 | 37 |
| | | 2.08 | 9 | Very marked | | 12 | 7 | 52 to 182 | 210 |
| | 15 | 0.02 | 32 | Marked | 41 | | 14 | 162 to 146 | 31 |
| Dog 21..... | | 0.67 | 11 | Marked | | 29 | 8 | 106 to 122 to 86 | 30 |
| | | 2.00 | 9 | Marked | | 17 | 7 | 101 to 151 | 101 |

* All doses greater than 2 cc. adrenalin 1 : 100,000 were actually injected into the animal in a 1 : 10,000 concentration, but to make the dose comparable in the tables they are given in terms of 1 : 100,000 dilution; 5 cc. adrenalin 1 : 100,000 in the table was really 0.5 cc. adrenalin 1 : 10,000 in the experiment. This applies to both tables.

## TABLE 2

*A comparison of the effects of adrenalin on different organs in the same individual*

| ANIMAL | WEIGHT | AMOUNT OF ADRENALIN INJECTED CUBIC CENTIMETERS OF 1:100,000 DILUTION PER KILOGRAM OF BODY WEIGHT | EFFECT ON INTESTINE | | | | EFFECT OF SPLEEN | | EFFECT ON KIDNEY | | EFFECT ON BLOOD PRESSRUE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Constriction | | Dilatation | | | | | | | |
| | | | Magnitude of constriction | Duration of constriction | Magnitude of dilatation | Duration of dilatation | Magnitude of change | Duration of change | Magnitude of change | Duration of change | Change in milli meters of mercury | Duration of change |
| | *kgms.* | | | *seconds* | | *seconds* | | *seconds* | | *seconds* | | *seconds* |
| Dog 11 | 15 | 0.03 | None | | Small | 80 | Small constriction | Persistent | | | 152 to 147 | 45 |
| | | 0.27 | None | | Marked | 105 | Marked constriction | 230 | | | 174 to 187 to 142 | 87 |
| | | 0.67 | Small | 15 | Very marked | 120 | Very marked constriction | 525 | | | 161 to 175 to 150 | 112 |
| Cat 13 | 2.9 | 0.07 | Small | 83 | | | | | Marked constriction | 80 | 132 to 140 to 109 | 67 |
| | | 0.35 | Small | 172 | | | | | Very marked striction | 210 | 80 to 92 to 68 | 115 |
| | | 1.72 | Marked | Persistent | | | | | Very marked constriction | 285 | 106 to 213 | 63 |
| Dog 21 | 15 | 0.01 | No effect | | | | Marked dilatation | 50 | | | 160 to 148 | 20 |
| | | 0.07 | None | | Small | 130 | Small dilatation followed by marked constriction | | | | 154 to 128 | 85 |
| | | 2.00 | Marked | 17 | Marked | 210 | Marked dilatation | 160 | | | 102 to 152 | 125 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog 22 | 25 | 0.02 | No effect | | | | Small dilatation | 48 | | | 132 to 123 | 44 |
| | | 0.20 | Small | 28 | None | | | | | | | |
| | | 0.60 | None | | Marked | 285 | Marked constriction | Persistent | | | 127 to 90 | 131 |
| | | | | | | | Marked constriction followed by small dilatation | 28 | | | 122 to 134 to 110 | 160 |
| | | 1.00 | Small | 18 | Very marked | 312 | Small constriction followed by persistent dilatation | | | | | |
| Dog 23 | 21.5 | 0.02 | Barely perceptible | 13 | | 115 | | | Marked constriction | 100 | 143 to 121 | 30 |
| | | 0.47 | Slight | 12 | Small | | | | Marked constriction | 102 | 148 to 131 | 128 |
| | | 0.93 | None | | Marked | Persistent | | | Marked constriction followed by dilatation | 42 | 144 to 125 to 224 | 97 |

In investigating the dilatation occurring in various organs in the body under the influence of different concentrations of adrenalin in the blood circulating through them, we hoped to gain some light on the vexed question of the existence of dilator fibres in the sympathetic nerves to the blood vessels. The existence of these has been denied by many authorities, notably by Brodie and Dixon (8), and by Cannon and Lyman (9). On the other hand, evidence deduced from experiments of widely differing character has been brought forward in support of the theory that dilator fibers are present in the blood vessels, and are sensitive to adrenalin in solutions too dilute to stimulate the endings of the constrictor fibres. Dale's experiments on the reversal of the effect of adrenalin by ergotoxine (10), are interpreted to this effect by him. The pioneer work of Brodie and Dixon (8) on the lung already cited, and the later results of Desbouis and Langlois (11) and especially those of Enid Tribe (12) seem to point in this direction, the dilatation observed by Park (13), Elliott (17), and Cow (15), in coronary vessels, and that in vessels of other organs by Pari (16) and by Ogawa (17), in response to adrenalin offer further proof of the possibility of the existence of vaso-dilator fibres in the sympathetic system. On this subject our experiments have the value only of a negative finding, but as far as they carry us we have found no evidence of a direct stimulation of vaso-dilator endings by adrenalin, in any concentration approaching that which occurs under physiological conditions.

## SUMMARY

1. Small doses of adrenalin cause constriction of the vessels of intestine, of kidney, and generally also of spleen.

2. The minimal dose necessary to produce this constriction is in much the same order of magnitude as that required to cause a fall in blood pressure, but it is not necessarily identical with it, nor is it the same for every organ in the same animal.

3. Increase of the dose of adrenalin causes in all cases marked dilatation of the intestine. This dilatation is brought about by doses materially less than those which are necessary to cause a rise in general blood pressure.

4. This dilatation in the intestine is under control of the central nervous system, and is done away with by severing connection with the central nervous system.

5. Adrenalin in the majority of cases has in all doses a constrictor

effect on the spleen; in some, minute doses cause first dilatation, which is the initial change of a series of rhythmical splenic waves.

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# THE MECHANISM FOR VASODILATATION FROM ADRENALIN

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Up to the present, no satisfactory explanation of the vasodilatation resulting from adrenalin has been offered. That it might be of central nervous origin was indicated by the experiments of S. J. and C. Meltzer (1) in the dilatation of the vessels of the rabbit's ear from subcutaneous injection of adrenalin. They obtained nothing but constriction if the vasomotor nerves were cut. However the explanation of dilatation from subcutaneous injection of adrenalin in the rabbit is not necessarily the same for the cat because no dose of adrenalin causes a fall of blood pressure in the rabbit as it does in the cat.

Dale's (2) theory that small amounts of adrenalin stimulate vasodilator endings while larger amounts bring vasoconstrictors into play, is not supported by the observation that small amounts of adrenalin cause constriction while larger amounts cause dilatation of the intestine of the dog and cat (3). Nor does the hypothesis of Cannon and Lyman (4) satisfy the facts observed in the differential action of adrenalin (5). They attributed the two effects, vasodilatation and vasoconstriction to opposite actions according to the state of the muscle—relaxation when tonically shortened, contraction when relaxed.

Finally the work of Gruber (6) suggests that the central nervous system is involved in the dilatation from adrenalin. He found that doses of adrenalin which caused dilatation in the normal limb of the cat produced constriction when the nerves were cut.

In attempting to explain the dilatation from adrenalin in the intestine of the cat and dog we (3) found that if the splanchnics were cut constriction replaced dilatation. This observation led us into a study of the part played by the central nervous system in adrenalin vasodilatation. The following investigation is the result.

Ether anaesthesia was used in all experiments, except a few in which anocain was injected. Blood pressure in all except the anocain experi-

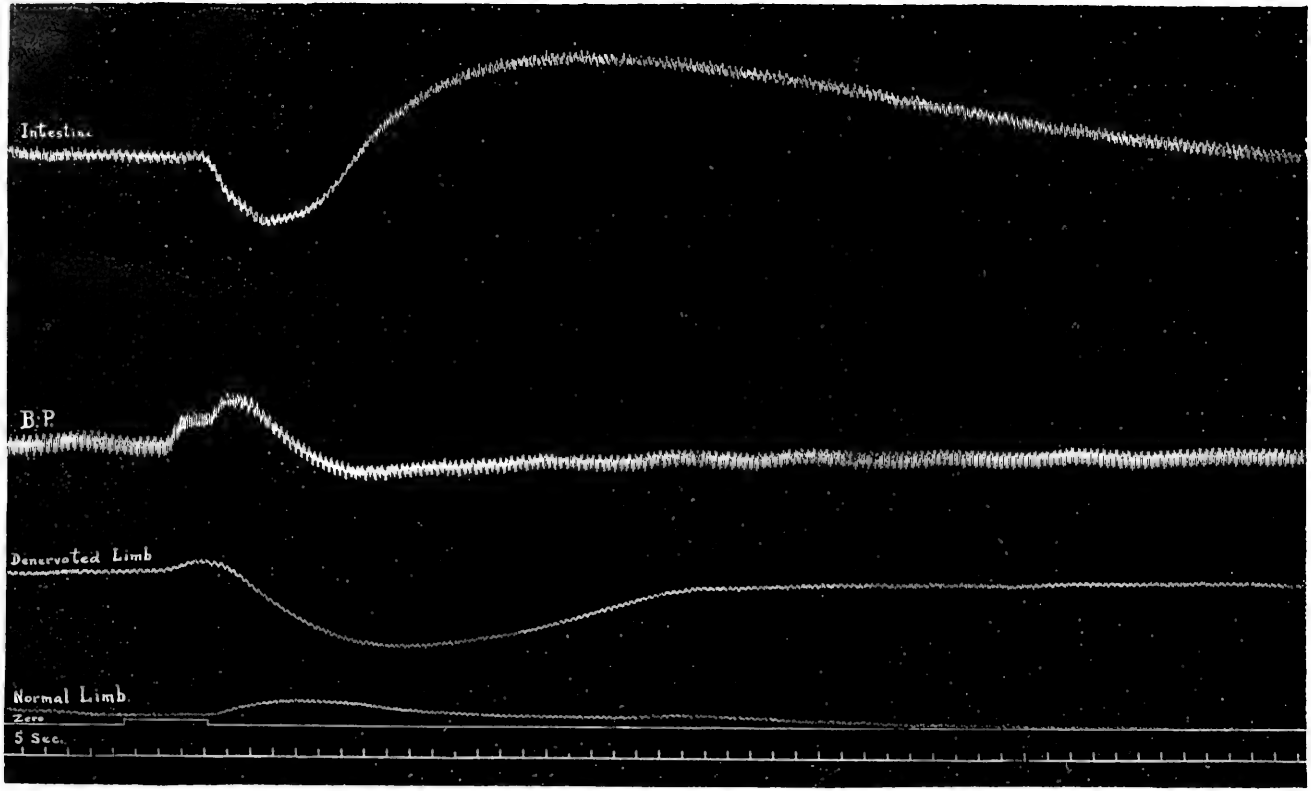


Fig. 2. Same as figure 1 in response to 2.0 cc. adrenalin, 1:100,000. (Reduced ½.)

0.025 cc. of adrenalin 1 : 1,000,000 caused a fall of blood pressure from 125 mm. to 120 mm., while 0.2 cc., 1 : 1,000,000 not only produced a fall of blood pressure from 127 mm. to 93 mm., but brought about constriction in both normal and denervated limbs. However, there is this to be said about these exceptional cases: the normal limb usually either gives no constriction or else much less constriction as compared to the denervated limb. So although there may be no positive dilatation there must be enough in the normal limb to lesson or else obliterate the local constrictor effect of adrenalin.

The following is a typical experiment of the ten which gave positive dilatation:

*Cat, weight 3.3 kgms. (See figs. 1 and 2)*

| DOSE OF ADRENALIN CC. 1:100,000 | BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY | NORMAL LIMB | | | | DENERVATED LIMB | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dilatation | | Constriction | | Dilatation | | Constriction | |
| | | Amount | Duration | Amount | Duration | Amount | Duration | Amount | Duration |
| | | *cc.* | *seconds* | *cc.* | *seconds* | *cc.* | *seconds* | *cc.* | *seconds* |
| 0.2 | 118–127– 96 | 0.13 | 95 | None | | 0.18 | 35 | None | |
| 0.4 | 118–131–100 | 0.33 | 178 | None | | 0.16 | 31 | None | |
| 0.6 | 122–138–100 | 0.5 | 325 | None | | 0.23 | 28 | 0.1 | 47 |
| 1.0 | 124–141–110 | 0.5 | | None | | 0.25 | 13 | 0.67 | |
| 1.5 | 127–153–117 | 0.34 | | None | | 0.15 | 11 | 0.9 | 275 |
| 2.0 | 127–157–113 | 0.13 | | None | | 0.10 | 9 | 1.4 | |
| 0.5 (1 : 10,000) | 127–196– 89 | 0.13 | 18 | 0.63 | 74 | 0.12 | 5 | 1.45 | 265 |

The dilatation in the denervated limb was passive because it occurred at the same time that the blood pressure began to rise and lasted no longer than the rise. This dilatation was cut short or obliterated with larger doses of adrenalin. Dilatation in the normal limb came later than in the denervated limb and lasted as long as the blood pressure was below normal.

This difference in reaction between the denervated and normal limbs is not due, as Gunning (8) suggests, to extreme dilatation of the denervated limb resulting from the absence of vasoconstrictor impulses. A depressor substance obtained from ox pituitaries was injected into the animal that produced figures 1 and 2. The dilatation of the denervated limb was as great as that in the normal limb, showing that the limit had not been reached.

In one dog (17 kgm.) a plethysmograph was placed so as to include a hind limb just above the ankle. Adrenalin was injected into the circulation. As a result the limb was constricted as much as 0.66 cc. in one instance and as little as 0.3 cc. in another. Without disturbing the plethysmograph both femoral and sciatic nerves to the enclosed limb were then severed. The same amount of adrenalin (0.2 cc., 1 : 10,000) was injected as before with a resulting constriction of 1 cc. or more in every instance. Moreover the duration of constriction in the dener-

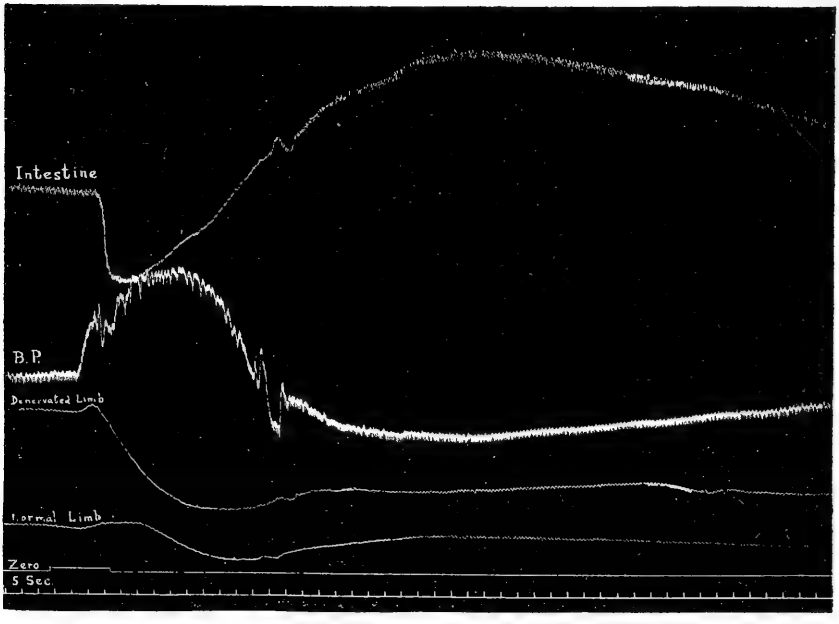


Fig. 3. Same as figure 1 in response to 0.5 cc. adrenalin, 1:10,000. (Reduced $\frac{2}{3}$.)

vated limb was almost twice as long as that before cutting central nervous connection (fig. 4). The greater and more prolonged constriction, after the nerves were cut, was no doubt due to absence of dilatation from central nervous influence. This explanation also probably applies to one of the cats which failed to give dilatation in the normal limb, for the constriction in the normal limb was always decidedly less than in the denervated limb.

We attempted to demonstrate the necessity of central nervous connection for dilatation from adrenalin in another way. The dose which

gave the maximum fall in blood pressure was determined. A number of injections were made so that the average might be found. After cutting the nerves to the limbs the percentage fall in blood pressure from

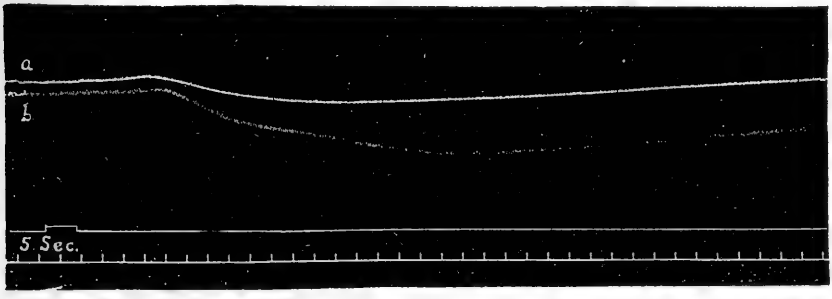


Fig. 4. Increase of constriction in a dog's foot by destroying central nervous connection. *a*, Before cutting nerves; *b*, after cutting nerves (0.2 cc. adrenalin, 1:10,000; weight 17 kgms.). (Reduced ½.)

the same dose was ascertained. According to the evidence just submitted the percentage fall should decrease with every limb denervated.

In the first animal, a cat weighing 1.3 kgm., 0.2 cc. adrenalin, 1:100,000 caused a fall in blood pressure of 18.7 per cent (from 123 mm.). After cutting the left brachial plexus the same dose produced a fall in blood pressure of 12.2 per cent, (from 114 mm.). Cutting the right brachial plexus reduced the fall to 9 per cent (from 100 mm.).

A cat weighing 2.3 kgms. responded to 0.1 cc. adrenalin, 1:100,000 by an 8 per cent fall in blood pressure (from 150 mm.), and to 0.3 cc. adrenalin, 1:100,000 by an average fall of 16.6 per cent (4 injections). When both brachial plexuses and both femoral and sciatic nerves had been cut the percentage fall from 0.1 cc. adrenalin, 1:100,000 was 2.7 while from 0.3 cc. adrenalin, 1:100,000 the average fall (4 injections) was 9.3 per cent (from 112 mm.), (see fig. 5).

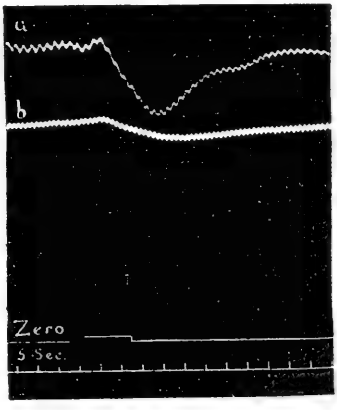


Fig. 5. Blood pressure reactions to adrenalin (0.3 cc., 1:100,000) before and after cutting the nerves to the limbs. *a*, Before; *b*, after. Cat weighing 2.3 kgms. (Reduced ½.)

The above doses were so small that the intestine would scarcely contribute to the fall in blood pressure. After dilatation in the limbs had been prevented by destroying the central nervous connection there still remained the important back, chest, shoulder and hip muscles, which easily accounts for the large percentage fall still obtained. On the whole, this evidence tends to support our plethysmographic proof of a central nervous function in adrenalin blood pressure fall.

### *2. By perfusion experiments*

A conclusive method of proving that the dilatation from adrenalin is due to a central nervous mechanism was devised. The part to be studied, limb or intestine, was cut off from the body circulation and perfused with warm oxygenated Ringer's solution. The nerves to this part were left intact. Dilatation of the part was ascertained by either the variation in outflow of the perfusing fluid or by means of a plethysmograph. Injections of adrenalin were then made into the body circulation and their effect upon the organ in question noted.

The prefusion fluid, oxygenated Ringer's solution or defibrinated blood mixed with Ringer's solution, was warmed by passing it through a tube enclosed in a cylinder through which warm water circulated. The apparatus was a modification of that described by Brodie and Cullis (9). A small thermometer inserted into the solution, by means of a T-tube, just before it entered the perfusion cannula enabled us to know the temperature as it started through the organ. Pressure was produced by compressed air led into the bottle containing the perfusion fluid. The pressure was controlled by means of a mercury valve.

The temperature of the liquid as it actually entered the organ was maintained in the neighborhood of 37°C. The pressure was usually somewhat below the general blood pressure.

To perfuse an intestinal loop without injuring its nerves large animals were almost necessary. A loop of intestine of a dog weighing 15 kgms. was perfused with a mixture of defibrinated blood and warm Ringer's solution after the circulation had been entirely cut off. The perfusion pressure was 120 mm. of mercury. The intestine was then enclosed in an oncometer. Injection of 0.5 cc. adrenalin, 1:10,000 into an external jugular vein caused a rise and fall in the blood pressure (136 mm. to 156 mm. to 93 mm.) and a dilatation in the intestinal loop which could in no possible way be reached by the adrenalin itself. A larger injection of adrenalin 2.0 cc., 1:10,000, caused a rise in blood pressure (97

mm. to 172 mm.) and a marked dilatation of the intestine. The dilatation of the intestine was accompanied by an increased outflow of the perfusion fluid.

We next prefused one hind limb of another dog (5.4 kgms.). The perfusion fluid was forced into the femoral artery and any change in the limb vessels was determined by measuring the outflow from the femoral vein. An injection of 0.2 cc. adrenalin, 1:10,000 caused an increase in the outflow from the femoral vein. In order to cut off the rest of the limb from the body circulation nearly all of the muscles were cut down to the bone, care being taken to leave the nerves intact. The same dose of adrenalin produced a decrease in the outflow. Apparently some adrenalin was still reaching the limb thus causing constriction and the vasodilator mechanism was perhaps inhibited by the vigorous operation of cutting the muscles.

The intestine and limb of a third dog (23.5 kgms. in weight) were perfused. The intestine employed was 20 cm. long. It could not be influenced directly by the adrenalin injected as it was entirely cut off from the body circulation; 0.7 cc. adrenalin, 1 : 10,000, injected into the jugular vein caused a dilatation of 0.28 cc. in the perfused intestinal loop; 3 cc. adrenalin, 1:10,000 into the general circulation produced a dilatation of 0.88 cc. in the loop. There was also a measurable increase in the venous outflow from the intestine.

One hind limb of this dog was included in a plethysmograph. The cylinder was large enough to include a major portion of the limb. Before its circulation was interfered with, 0.5 cc. adrenalin 1:10,000 injected into the general circulation caused a dilatation of 0.86 cc. (see fig. 6). Cannulae were inserted into the iliac artery and vein just below the bifurcation. The aorta was clamped considerably above the iliacs in order to prevent access of blood to the perfused limb by anastomoses. Ringer's solution at a temperature of 37°C. entered the limb by way of the iliac artery under pressure of 100 mm. of mercury. The same amount of adrenalin as before was injected into the general circulation. The first time the perfused limb dilated 0.68 cc. while at the second injection the expansion was 0.90 cc. In other words the amount of dilatation was about the same when circulatory connection was destroyed as when it was intact (see figs. 7 and 8).

The amount of dilatation varied with the dose; 0.25 cc., 1:10,000 gave 0.27 cc., while 1 cc., 1:10,000 gave 1.59 cc. After the limb had been perfused one and one-half hours, 1.0 cc. adrenalin, 1:10,000 into the jugular vein gave only 0.72 cc. dilatation. Another interesting

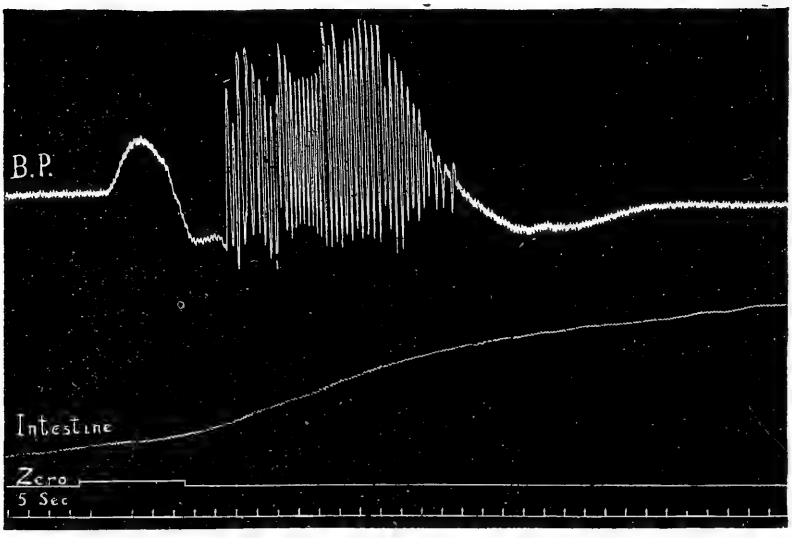


Fig. 6. Dilatation of a perfused loop of intestine, which has no connection with the body circulation, in response to adrenalin (3.0 cc., 1:10,000) injected into the jugular vein. Nerves to intestine undisturbed. Dog weighing 23.5 kgms. (Reduced $\frac{1}{2}$).

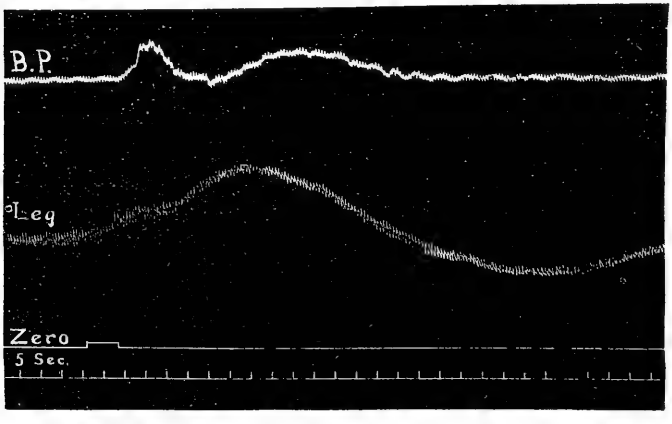


Fig. 7. Dilatation of a dog's hind limb in response to 0.5 cc. adrenalin, 1:10,000. Dog weighing 23.5 kgms. (Reduced $\frac{1}{2}$.)

fact was the persistence of dilatation in the perfused limb. The intact limb returned to normal in sixty-five seconds when a dose of 0.5 cc. adrenalin, 1:10,000 was given, while the same limb perfused returned very slowly or not at all after a similar dilatation from adrenalin.

Thus we have proven both by cutting the nerves and by destroying the circulatory connection that the dilatation from adrenalin, in the intestine and in the limb, is due to some central nervous mechanism.

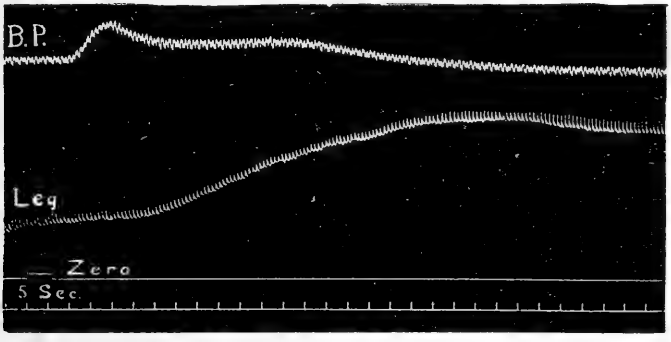


Fig. 8. Response of same limb as in figure 7, after connection with the body circulation has been destroyed. Perfused with oxygenated Ringer's solution. Nervous connection intact. Adrenalin (0.5 cc., 1:10,000) injected into jugular vein. (Reduced $\frac{1}{2}$.)

## INHIBITION OF THE ADRENALIN VASODILATOR MECHANISM

Although only about 5 per cent to 10 per cent of cats and dogs fail to give a fall in blood pressure with small doses of adrenalin, many of those animals are not normal in other ways. In a former study ( (5) p. 443) three out of five such cases were diseased or emaciated while a fourth had been intoxicated with alcohol a few hours before. More recently we have investigated a case (cat weighing 2.4 kgms.), in which there was either only a slight fall or else no fall in blood pressure with doses of adrenalin as small as 0.1 cc. or 0.2 cc., 1:1,000,000. The intestine was placed in an oncometer and gave dilatation after the injection of 0.5 cc. adrenalin, 1:10,000 into the circulation. So in spite of the fact that there may be no definite fall in blood pressure, dilatation of the intestine may occur.

These observations kept us on the lookout for other conditions which might inhibit the adrenalin vasodilator mechanism. We found a cat

weighing 4.3 kgms. which gave a fall in blood pressure of only 6 mm. (from 113 mm.) with 0.6 cc. adrenalin, 1:100,000. At the same time there was little or no effect on the volume of the normal limb. Associated with this poor response was a very dark venous-colored blood in the arteries. There had been no other signs of asphyxia. A plug of mucus in the trachea was found. Several minutes after the restoration of ventilation by removal of the mucus 0.5 cc. adrenalin, 1:100,000 produced a fall in blood pressure of 38 mm. from 140 mm. with dilatation of the normal limb. In this animal partial asphyxia seemed to produce incomplete inhibition of the adrenalin vasodilator mechanism.

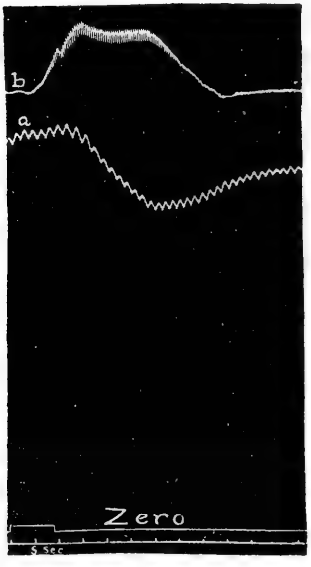


Fig. 9. Reversal of blood pressure response to 0.5 cc. adrenalin 1 : 100,000, after injecting anocain. *a*, Before injecting anocain; *b*, after injection of anocain. Cat weighing 2.2 kgms. (Reduced $\frac{2}{5}$.)

We had occasion to employ anocain (manufactured by Wingate Chemical Company, Ltd., Montreal) as a local anaesthetic in taking blood pressure from a dog's femoral artery. Injections of adrenalin in doses which in other animals gave a fall in blood pressure caused either no effect or only a rise in blood pressure. In order to determine whether the abnormal response was due to anocain a cat (weight 2.2 kgms.) was gently fastened to the board without its being alarmed. Then a solution of anocain (0.05 gram) was injected into the skin and later the muscles on either side of the femoral artery. Adrenalin, 1:100,000 was injected into the femoral vein. Blood pressure was registered from the femoral artery; 0.1 cc. caused a rise of 15 mm. from 180 mm.; 0.5 cc. increased the blood pressure 26 mm. from 192 mm., (see fig. 9). Following this the cat was placed under the influence of ether. Thirty minutes after the above reaction with anocain anaesthesia, 0.1 cc. adrenalin produced a fall of 10 mm. in blood pressure (from 175 mm.) while 0.5 cc. decreased the blood pressure 24 mm. (from 173 mm.).

One hour after anocain had been given, 0.5 cc. adrenalin produced a fall of 33 mm. (from 175 mm., see fig. 9). Anocain was again given intramuscularly after which adrenalin produced nothing but a rise in blood pressure.

After sufficient time had elapsed the fall in blood pressure again returned upon the injection of the usual dose of adrenalin.

This experiment was repeated in another cat with similar results. We would conclude that anocain inhibits the action of the adrenalin vasodilator mechanism.

There is some evidence that dilatation of a limb may be prevented by afferent impulses from that part. Many of the experiments which gave no dilatation from adrenalin in the normal limb were performed in a cold laboratory (15°C. or less). Although the operating table was warmed, the limbs were never as warm as the back of the animal. Because the temperature was much lower than in most of our successful experiments we thought that it might be the cause of a local inhibition of dilatation in the limb. Dilatation from adrenalin was occurring some where in the animal for there was a marked fall in blood pressure with the usual dose. The intestine was not dilating because the dose of adrenalin was below the intestinal dilatation threshold, moreover the loop of the intestine in the oncometer was constricting. We assumed that the dilatation must take place in the muscles which were kept warm, such as the back muscles. To investigate this assumption we warmed the normal limb with heat from an electric lamp. After the limb had ceased dilating from the artificial heat the usual dose of adrenalin injected into the general circulation caused an increase in volume of the limb. Previously the same doses of adrenalin produced either no effect or else constriction in the same limb.

It seems also that cutting the muscles in a limb may inhibit the dilatation from adrenalin. In one of the first perfusion experiments mentioned above, the response to the usual dilator-dose of adrenalin was changed to constriction by cutting part of the muscles of the thigh although the nerves were not injured.

## BEHAVIOR OF THE ADRENALIN VASODILATOR MECHANISM

Simultaneous records of the volume changes of the intestine, normal hind limb and denervated hind limb together with the blood pressure in response to adrenalin, were obtained from thirteen cats. Whether the threshold dose for constriction in the normal limb was also the threshold dose for dilatation in the intestine could be answered in this manner.

Our apparatus showed that the threshold dose for dilatation in the limb was a little larger than the smallest dose causing a blood pressure

fall, while the threshold dose for intestinal dilatation was usually four or five times as large as the threshold dose for the limb. Nor does the intestinal dilatation reciprocate the action of the normal limb for the latter usually continues to dilate some time after the threshold dose for dilatation of the former has been reached. Moreover there is an important distinction in the behavior of the limb and intestine. If we look at the effects on these respective parts with increasing doses we see that although the dilatation of the limb increases with increasing doses up to a certain point, beyond that the dilatation becomes less and less until constriction results. On the other hand constriction is the first adrenalin effect in the intestine, while with increasing doses dilatation appears and becomes larger and larger but is never replaced by constriction after the maximum is reached. Stated in another way, small doses of adrenalin act on the adrenalin vasodilator mechanism for the limb while large doses act on the adrenalin vasodilator mechanism for the intestine. Figures 1, 2 and 3 picture the results with increasing doses of adrenalin. The following experiment illustrates the foregoing statements:

*Cat, weight 3.3 kgms.*

| DOSE OF ADRENALIN 1:100,000 | BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY | NORMAL LIMB | | DENERVATED LIMB | | INTESTINE | |
|---|---|---|---|---|---|---|---|
| | | Dilatation | Constriction | Dilatation | Constriction | Constriction | Dilatation |
| *cc.* | | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 0.2 | 126–135–107 | 0.13 | | | 0.2 | 0.23 | |
| 0.6 | 124–140–106 | 0.26 | | 0.15 | 0.6 | 0.5 | |
| 1.0 | 125–141–112 | 0.25 | | 0.10 | 0.55 | 0.18 | 1.0 |
| 2.0 | 132–153–119 | 0.20 | | 0.10 | 0.85 | 0.63 | 1.22 |
| 0.5 (1:10,000) | 127–196– 89 | 0.13 | 0.63 | 0.12 | 1.45 | 1.22 | 2.34 |

## AN INTERPRETATION OF THE ERGOTOXINE REVERSAL OF ADRENALIN ACTION

Dale (10) has shown that ergotoxine inhibits the action of the endings of constrictor fibers. Instead of assuming as Dale suggests that the fall in blood pressure from pressor doses of adrenalin after ergotoxine is due to stimulation of vasodilator endings, in the light of our present research we would interpret it in another way. We suggest that the fall is due to stimulation of the central vasodilator mechanism by adrenalin and failure to stimulate the constrictor endings because of their

paralysis by ergotoxine. While constrictor endings function normally their total effect is greater than that of the vasodilator mechanism when pressor doses of adrenalin are injected. In a cat we were able to obtain evidence in support of this interpretation. We first determined the response of normal limb, denervated limb and intestine to adrenalin. Ergotoxine phosphate was given until the denervated limb which previoulsy had given constriction, gave no response to adrenalin. The normal limb still responded by dilatation. With larger doses of adrenalin the intestine dilated also.

There is additional evidence that our interpretation of ergotoxine reversal is correct, in the failure to obtain a reversal in the rabbit after ergotoxine (10). Unpublished work from this laboratory shows that the rabbit does not possess the adrenalin vasodilator mechanism; that is, adrenalin will not cause vasodilatation in the limb or in the intestine.

## LOCATION OF THE ADRENALIN VASODILATOR MECHANISM

We have proven by experiment that destruction of the cerebrum does not prevent the vasodilatation from adrenalin.

A cat (2.8 kgms.) gave a fall in blood pressure of 29 mm. from 150 mm. with 0.2 cc. adrenalin, 1:100,000. After removal of the cerebrum the same dose produced a fall of 22 mm. from 114 mm. Further investigation is being carried on as to the location of the adrenalin vasodilator mechanism.

We have been able to show that the central nervous system is necessary for active dilatation resulting from adrenalin. Just how this mechanism is brought into action, it is impossible to say at the present time. The use of such a mechanism is obvious in case of muscle, but the object of vasodilatation in the intestine is not evident.

## SUMMARY

1. Adrenalin vasodilatation in the intestine and limb is prevented by cutting the nerves to these organs.

2. A perfused limb or intestinal loop with nerves intact but without circulatory connection will dilate when the appropriate amount of adrenalin is injected into the general circulation.

3. Ill health, asphyxia, alcohol or anocain may inhibit the action of the adrenalin vasodilator mechanism.

4. Afferent impulses such as result from cooling or from cutting the muscles of a limb may impair or inhibit the adrenalin dilator reaction in that part.

5. The dilatation from adrenalin in a norma limb is produced by smaller amounts of adrenalin than those which cause intestinal dilatation.

6. Limb dilatation is replaced by constriction if the adrenalin is in sufficient quantity.

7. Intestinal dilatation is never replaced by constriction if the dose is above the dilatation threshold.

8. Ergotoxine reversal of adrenalin pressor effects is accounted for by paralysis of the vaso constrictors which mask the adrenalin vasodilator mechanism when large doses of adrenalin are used.

9. The adrenalin vasodilator mechanism is not located in the cerebrum.

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# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES

No. 16: ADRENALIN VASODILATOR MECHANISMS IN THE CAT AT DIFFERENT AGES, BY FRANK A. HARTMAN and LESLIE G. KILBORN

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# University of Toronto Studies

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# ADRENALIN VASODILATOR MECHANISMS IN THE CAT AT DIFFERENT AGES

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Received for publication November 13, 1917

It has been established by a number of investigators (1), (2) that in the cat the normal response of the vascular system to small doses of adrenalin is such as to cause a fall in blood pressure. This effect is accomplished by a dilatation in the blood vessels in the muscles (3). Moreover the dilatation is controlled by a central nervous mechanism (4).

We accidentally discovered that adrenalin fails to produce a fall in blood pressure in young kittens with any dose, however small. The only reaction given is a rise. Inasmuch as the fall in blood pressure is controlled by a central nervous mechanism it seemed possible that the failure of this mechanism to develop at an early age might account for the reaction in young kittens.

With the hope of throwing more light on the nature of the adrenalin vasodilator mechanism we have made a study of cats at different ages.

The methods employed in this research were similar to those used in previous researches (4) but with the following modifications: Much smaller bellows than those used in adult cats were found advantageous in registering volume changes of the limb or intestine of kittens. Bellows with a base 26 mm. by 13 mm. were used in a majority of the experiments, while occasionally in the youngest kittens a smaller bellows with a base 17 mm. by 10 mm. was tried. All animals were under the influence of ether.

When the age of the kittens was unknown, it was necessary to make an estimate from the animal's weight. These estimates were based upon the weights of five kittens, whose ages were known, ranging from 0.3 kgm. to 1.3 kgm. in weight. The ages so determined are close enough for our purpose because the earliest occurrence of the adrenalin vasodilator mechanism is unquestionably variable.

BLOOD PRESSURE REACTION AT DIFFERENT AGES

A study of the blood pressure responses might be expected to give us an idea of the age at which the adrenalin vasodilator mechanism begins to appear. We therefore sought to answer our problem by means of the blood pressure reaction.

The youngest kittens employed were of known age (three weeks) and weighed 0.3 kgm. and 0.32 kgm. respectively. The threshold for blood pressure response to adrenalin was high in both cases. In the first, 0.1 cc., 1:100,000 caused a rise from 46 mm. to 49 mm., while less than that produced no effect. Even larger doses produced a smaller percentage rise than that from proportional doses in older kittens, although the duration of the effect might be as long (see *a*, fig. 1).

Eight older kittens weighing from 0.6 kgm. to 0.67 kgm. (about eight weeks of age) possessed a lower threshold for adrenalin, in some instances being as low as 0.2 cc., 1:1,000,000. In only two of these animals was there an occasional fall of blood pressure succeeding the rise. When it did occur it was small in amount. A depressor effect at this age was exceptional (see *b*, fig. 1). Kittens even older failed to give a fall in blood pressure with adrenalin. Seven individuals weighing respectively 0.72 kgm., 0.75 kgm., 0.9 kgm., 0.9 kgm., 0.95 kgm. and 1.05 kgm. (estimated ages, nine to eleven weeks), gave a rise without a fall in every injection, however small.

On the other hand animals weighing 1 kgm. or more usually gave a rise and fall in blood pressure with small doses, although repeated injections in the same animal might not always do so (*c* and *d*, fig. 1). It might be suggested that the failure of a depressor reaction in these cases was due to the easy fatigue of an incompletely developed mechanism. Kittens of the following weights gave the depressor reaction: 1.0 kgm., 1.0 kgm., 1.1 kgm., 1.3 kgm.

As the animals became older the pressor effects from small doses of adrenalin became gradually less and less while the fall in blood pressure became greater and more prolonged. Finally in the adult animal the rise became insignificant or in some cases a pure fall resulted (see fig. 1).

A study of the blood pressure reaction has shown that the depressor response to adrenalin begins to appear at the age of eleven or twelve weeks. From that age onward the depressor response encroaches more and more upon the pressor effect until finally the latter may almost disappear, provided small doses of adrenalin are injected. This graded increase of the depressor response with the growth of the animal indicates a gradual development of the adrenalin vasodilator mechanism.

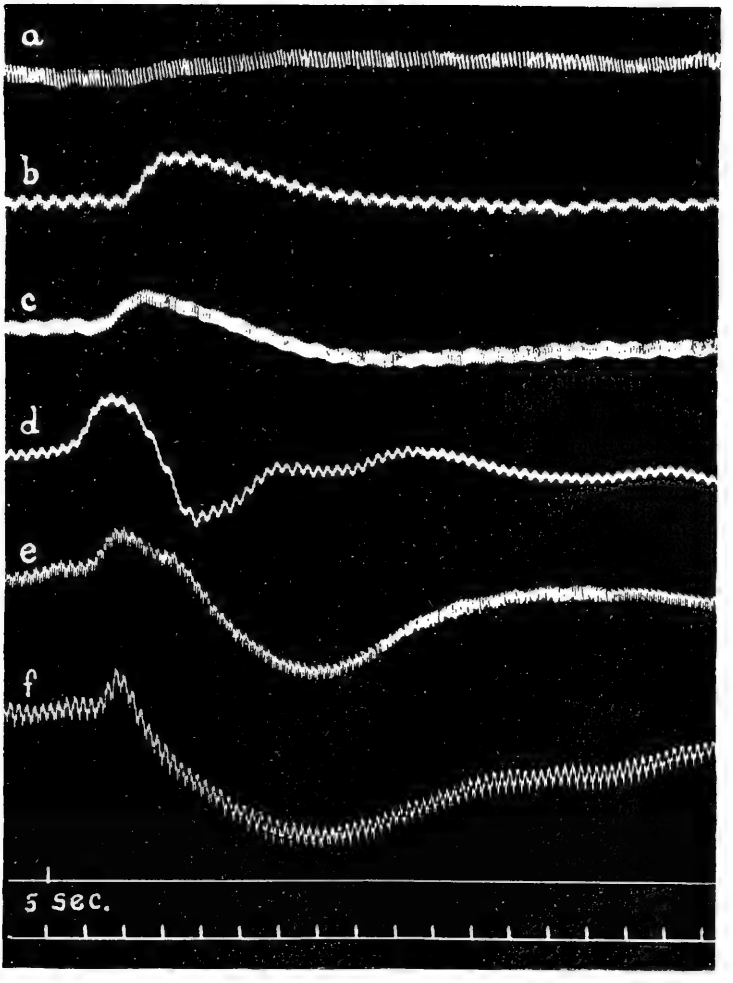


Fig. 1. Different types of blood pressure curves produced by adrenalin in cats of different ages.

| | WEIGHT | AGE | DOSE OF 1: 100,000 ADRENALIN | INITIAL HEIGHT OF BLOOD PRESSURE |
|---|---|---|---|---|
| | *kgms.* | *weeks* | *cc. per kgm. of body weight* | *mm. of mercury* |
| *a* | 0.30 | 3 | 1.67 | 60 |
| *b* | 0.62 | 8 | 0.16 | 67 |
| *c* | 1.0 | 11 | 0.20 | 119 |
| *d* | 1.3 | 14 | 0.076 | 170 |
| *e* | 1.8 | 24 | 0.17 | 150 |
| *f* | 2.8 | adult | 0.071 | 151 |

It seemed at first that we had settled the question as to the age at which the adrenalin vasodilator mechanism first appears by a study of the blood pressure reaction. But in work on adult cats, which is to be published soon, we found later that an adrenalin vasodilator mechanism might be acting in an animal although the blood pressure response was a rise. Therefore without a study of the volume changes in the organ concerned, we cannot be certain of our solution.

For reasons given in another research (4, p. 366) we are led to treat separately the adrenalin vasodilator mechanism for the limb and the intestinal adrenalin vasodilator mechanism. It might be well to briefly repeat those reasons. First, there is a difference of threshold, i.e., the mechanism for the intestine has a higher threshold on the aver-

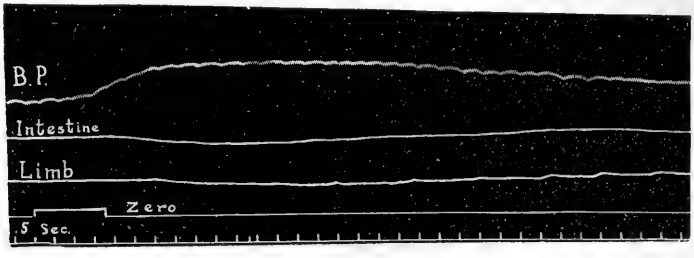


Fig. 2. Effect of 0.4 cc. adrenalin, 1:100,000 on the volume of the intestine and hind limb in a kitten three weeks old (0.32 kgm.). Smaller doses produced similar though less marked effects. (Reduced $\frac{1}{2}$.)

age than that for the limb. Second, a difference in reversal, i.e., no increase in the dose of adrenalin ever changes intestinal dilatation to constriction when once the dilatation threshold is passed, while such an increase does cause a reversal from dilatation to constriction in the limb. These observations suggest different types of mechanisms. We will therefore discuss them separately.

## THE ADRENALIN VASODILATOR MECHANISM FOR THE LIMB

Although we have employed the hind limb in this study, there is ground for assuming that its reaction (provided skin effects are negligible) represents the reaction for the skeletal muscle throughout the organism. We have therefore considered active adrenalin dilatation of the hind limb as proof of the presence of the adrenalin vasodilator mechanism for skeletal muscle.

We have sought for the presence of this mechanism in nine kittens at varying ages. The smallest to give undoubted evidence of its existence was nine or ten weeks old (0.85 kgm.) (see fig. 3). However the repeated injections of similar doses did not always produce active dilatation. This finding is parallel to the observation on the inconstancy of the depressor response in blood pressure in kittens first to show the reaction. Five younger kittens from three to eight weeks old gave no active dilatation of the limb. (See fig. 2.)

Two kittens about eleven weeks old (1 kgm.) reacted by limb dilatation more easily than did the nine-weeks-old kitten. As the animals

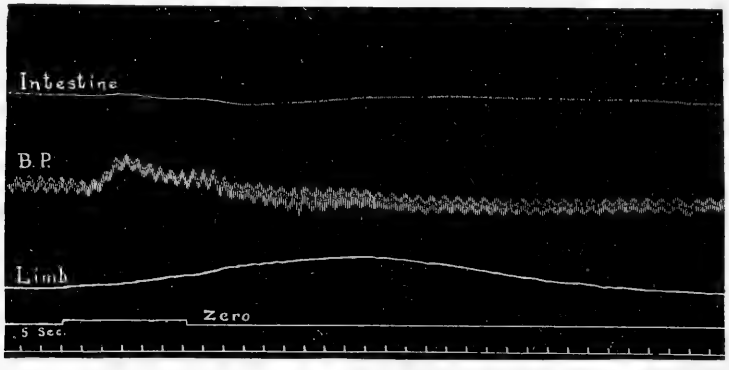


Fig. 3. Active vasodilatation in the limb of a kitten about nine weeks old (0.85 kgm.). Dose of adrenalin 1 cc., 1:100,000. (Reduced $\frac{1}{2}$.)

grew older the reaction was elicited with greater constancy. At six months the response resembled more that of the adult.

In general we may say that the limb mechanism begins to function at or possibly before the eleventh week. Inasmuch as the fall in blood pressure is due to the action of an adrenalin vasodilator mechanism for skeletal muscle, we should find that the depressor response of blood pressure and the active limb dilatation begin to appear at the same age. Within the limit of experimental conditions we have found this to be true.

### THE INTESTINAL VASODILATOR MECHANISM

Although the intestinal vasodilatation from adrenalin may contribute to the fall in blood pressure with doses that do not produce constriction in skeletal muscle, as soon as these doses are exceeded, intestinal vasodilatation merely subtracts from the pressor effects of the con-

stricting skeletal muscle. Therefore we cannot expect to throw much light on the blood pressure reaction by a study of the development of this mechanism. But because it seems to be of a different type we were anxious to compare its development with that of the limb mechanism.

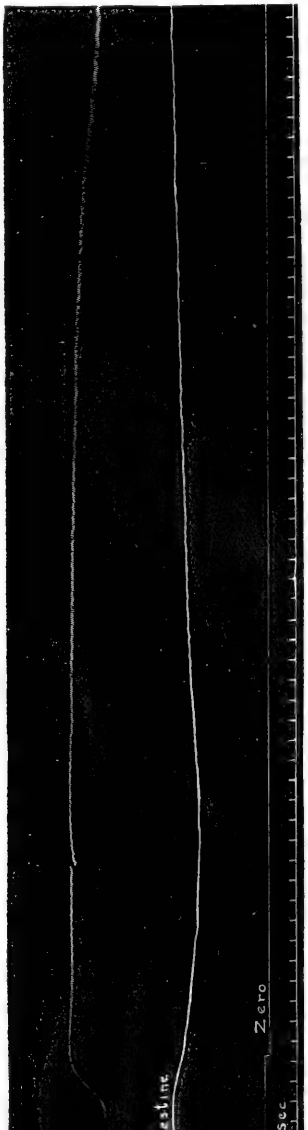


Fig. 4. Intestinal reaction to a large dose of adrenalin, 0.4 cc., 1:20,000 in a three-weeks-old kitten (0.32 kgm.). (Reduced ½.)

The volume changes in the intestine, resulting from the injection of adrenalin into the general blood stream, were observed in eleven kittens ranging in age from three weeks to six months. The amounts of adrenali ninjected varied from that just sufficient to give a response to massive doses. The absence of the intestinal adrenalin vasodilator mechanism was considered proven if massive doses failed to cause dilatation.

All kittens up to about eleven weeks of age (eight) failed to show the presence of an adrenalin vasodilator mechanism for the intestine. Three of about this age showed nothing but constriction, while a fourth gave a marked dilatation (see fig. 6). The character of the constriction differed somewhat with the age of the animal, younger kittens showing a more prolonged effect than older kittens (see figs. 4 and 5). This might be due to the vasodilator effects beginning to appear in the older kittens because we know that in adults the constriction is cut short by dilatation. At that stage the vasodilator mechanism is fully developed.

On account of differences already mentioned we have been led to consider the adrenalin vasodilator

mechanisms as of two different types. If this is true, the question arises as to whether the limb and intestinal adrenalin vasodilator mechanisms begin to function at the same age. This could best be answered by seeking for them in the same individual. Ten kittens were tested for the presence of both the limb and intestinal adrenalin vasodilator mechanisms. The youngest to show the presence of either mechanism was about nine weeks old. There was positive evidence of the presence of the adrenalin vasodilator mechanism for the limb, but absolutely no trace of the other mechanism. Two kittens about

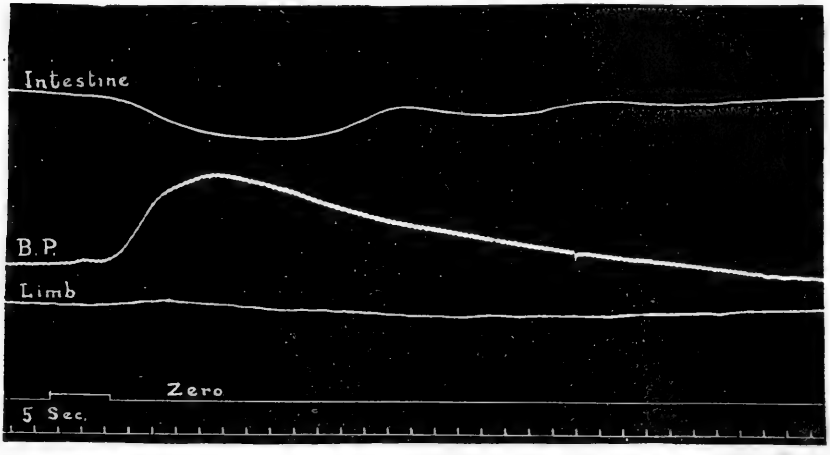


Fig. 5. Less prolonged intestinal constriction from a large dose of adrenalin (0.5 cc., 1:10,000) in an older kitten than in the previous figure. Age eight weeks, weight 0.67 kgm. (Reduced ½.)

eleven weeks of age gave active limb dilatation with adrenalin but absolutely no intestinal dilatation. We may conclude from these results that the two mechanisms may begin to function at different ages in the same individual. Moreover in every case so far noted (three) the adrenalin vasodilator mechanism for the limb functioned earliest. These observations lend support to the idea that the two mechanisms are of different types.

In conclusion we may ask: Why do the adrenalin vasodilator mechanisms develop so late in the life of the individual? Does it mean that the mechanism is one of the last to appear in the evolution of the cat? If so, it might be that they are specialized mechanisms occurring

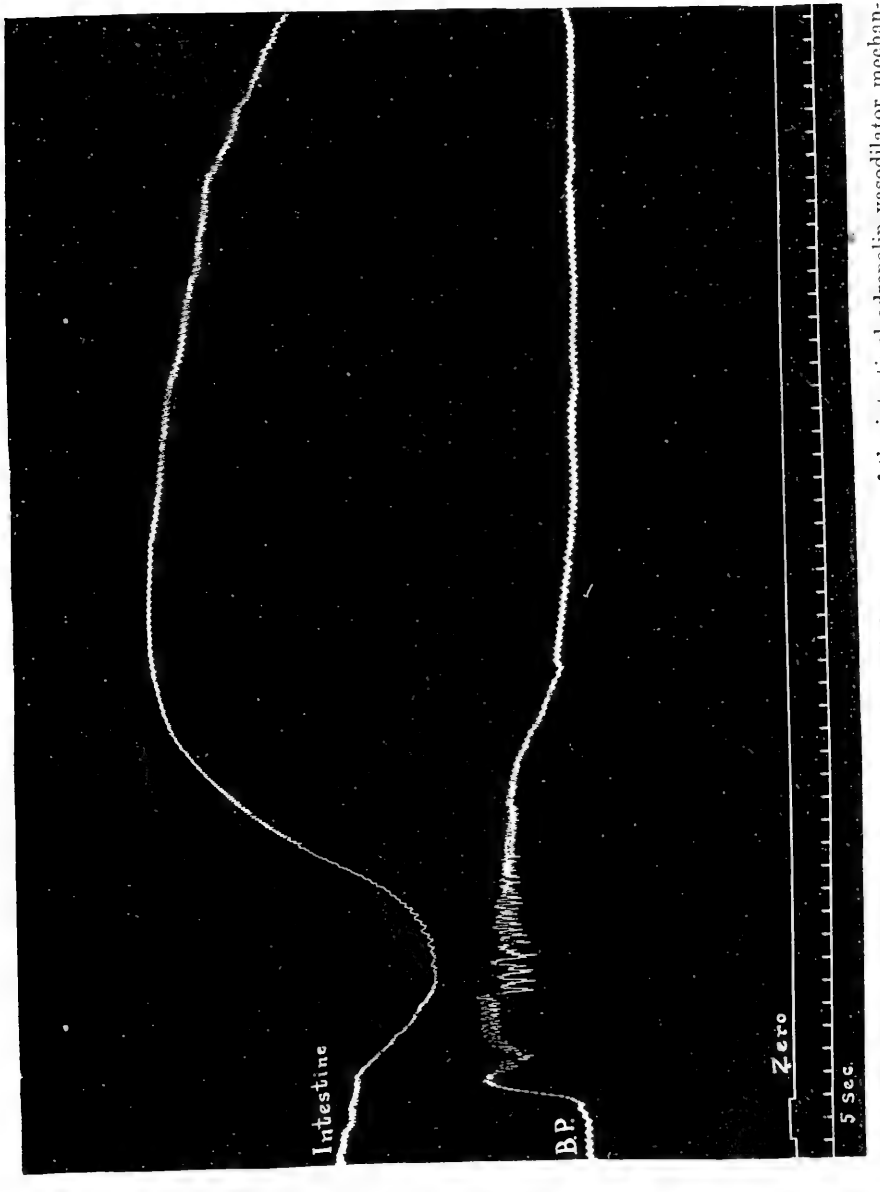


Fig. 6. The youngest kitten to give evidence of the presence of the intestinal adrenalin vasodilator mechanism. Age, eleven to twelve weeks. Weight, 1.1 kgm. Adrenalin injected, 0.5 cc., 1:10,000. (Reduced ⅔.)

only in the carnivora. (Their presence has been proven in the dog.) A systematic survey of the vertebrates for the presence of these mechanisms is in progress in this laboratory.

## SUMMARY

1. The smallest effective doses of adrenalin produce only a rise in blood pressure in young kittens.

2. The threshold for adrenalin blood pressure effects is high in young kittens, decreasing as they grow older.

3. The response to adrenalin of a fall in blood pressure begins to appear at about eleven weeks.

4. The increasing of the depressor effects from the slight fall succeeding a rise in younger animals to a marked almost pure fall in adults indicates a gradual development of the adrenalin vasodilator mechanism.

5. This fall in blood pressure seems to be due to vasodilatation in skeletal muscle, for the two begin to appear simultaneously in most instances.

6. The intestinal adrenalin vasodilator mechanism often develops later than the adrenalin vasodilator mechanism for the limb. This supports the view that the two mechanisms are of different types.

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# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES


No. 17: LOCATION OF THE ADRENALIN VASODILATOR MECHANISMS, BY FRANK A. HARTMAN, LESLIE G. KILBORN and LOIS FRASER

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# LOCATION OF THE ADRENALIN VASODILATOR MECHANISMS

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It has been demonstrated (1) that adrenalin vasodilatation in the limb can be produced by stimulation of other than peripheral structures. It has also been shown that intestinal vasodilatation from adrenalin involves either the collateral ganglia or the central nervous system. These facts were established, *a*, by cutting the nerves to the limb in the one case and by destroying the ganglia in the other; *b*, by perfusion experiments in which the organ is cut off from the body circulation and the nerves left intact, adrenalin being injected into the jugular vein.

Previous to the present research we had found evidence which indicated a difference between the type of mechanism causing vasodilatation in the limb, as an example of skeletal muscle, and that producing like effect in the intestine: viz., 1, small doses of adrenalin produce constriction in the intestine and dilatation in the limb while larger doses produce the reverse effect, i.e., dilatation in the intestine and constriction in the limb (1, p. 366); 2, greatly increasing the dose above that causing intestinal dilatation does not produce predominant constriction in the intestine; 3, the intestinal vasodilator mechanism develops later than the corresponding mechanism for the limb (2).

In the present research we have attempted to determine the location of these mechanisms.

The procedure followed was to destroy different portions of the brain and spinal cord, to remove the sympathetic ganglia or to destroy the dorsal root ganglia of the nerves from the organ investigated and then to ascertain the activity of the vasodilator mechanisms according to methods described in previous investigations (1).

*Removal of cerebrum and cerebellum.* A cat (2.8 kgm.) responded to 0.2 cc., 1: 100,000 adrenalin with a fall in blood pressure of 19.3 per cent (150 mm. to 121 mm.). Nine minutes after removal of the cere-

brum a similar dose of adrenalin produced the same percentage drop in blood pressure (114 mm. to 92 mm.) This indicates that the adrenalin vasodilator mechanisms are not in the cerebrum, at least those which control the vessels of skeletal muscle and are called into play by small doses of adrenalin. In order to confirm our conclusion in regard to the position of these, we studied the volume changes in the hind limb of one cat and one dog. Dilatation of the limb of the cat from adrenalin occurred after decerebration as well as before. A similar result was obtained in the dog after removal of both cerebrum and cerebellum.

Blood pressure changes, however, do not indicate the action of the intestine, therefore in order to discover whether the intestinal mechanism was present in the cerebrum the volumetric method was necessary. In both animals the intestinal dilatation thresholds were determined before decerebration. The cerebrum was destroyed in the cat, then the same dose of adrenalin was injected as before with like result, showing that the intestinal mechanism was not in the cerebrum. The dog had both the cerebrum and the cerebellum removed without interfering with the intestinal dilatation.

We are justified, therefore, in concluding that neither type of the adrenalin vasodilator mechanisms is present in the cerebrum or cerebellum.

*Destruction of the medulla.* Our next step was to destroy the medulla. This was done in those animals which had served for the cerebral experiments, and in some others in which the brain was pithed in one operation. (In either case the ether was immediately discontinued.) Destruction of the medulla always produced a reversal in the blood pressure response to adrenalin, in none was there a fall in blood pressure (four dogs and eight cats). It appeared, therefore, that the dilator mechanisms might be situated in this region of the central nervous system. A typical example is as follows:—before pithing the brain, 0.2 cc., 1: 100,000 adrenalin produced a 14 per cent fall in blood pressure in a cat (166 mm. to 152 mm.); twice the amount produced a 30 per cent fall. After pithing, the same doses of adrenalin produced 10 per cent and 32 per cent rises in blood pressure, respectively (from 78 mm.). One of us has shown (3), however, that a decrease in the blood pressure (e.g., by hemorrhage) is enough in itself to produce a similar result. The reversal in the reaction in this case then was not necessarily due to destruction of the dilator mechanisms. We sought an answer to this question by a study of the volume changes of the

organs. The dilatation of the limb muscles, recorded with the plethysmograph, in two dogs and four cats followed the curve of the rise in blood pressure and seemed to be passive. Intestinal volume changes were recorded in seven subjects (two dogs and five cats). In all but one the dilatation was active, independent of the rise in pressure.

In order to exclude all possibility of passive dilatations, the hind limb of a cat was perfused with warm oxygenated Ringer's solution through the common iliac artery. The abdominal aorta was clamped high up to prevent anastomoses. The vena cava was tied and an outlet made in the common iliac vein (1, p. 360). The response of the perfused limb to various doses of adrenalin injected into the general circulation was noted, after which the brain was pithed and the injections repeated. The resulting dilatation was in every case as marked as before. Similarly the perfused hind limb of a dog responded by dilatation as well after destruction of the brain as before. A perfused intestinal loop of a brainless dog dilated when adrenalin was injected into the general circulation.

*Cervical cord.* Having failed to locate the vasodilator mechanisms in the medulla or higher, their presence in the cord of the cervical region seemed very doubtful. The upper part of the central nervous system down to the thoracic cord was destroyed by pithing in a dog and a cat. The limb mechanism in the dog was active, as determined by the plethysmograph after pithing. The intestinal reaction of the dog was not studied but in the cat it was still present. It must be concluded that both mechanisms are below the cervical cord.

*Thoracic cord.* After destruction of the central nervous system as far down as the mid-thoracic region the adrenalin vasodilator mechanism for the intestine still worked in every case (four cats and one dog). It appeared, therefore, that the intestinal mechanism must be located below this region.

The cord was next pithed to the lumbar region in three cats and one dog. Adrenalin still caused intestinal dilatation in all cases, though in the dog the dilatation was not as marked as before. Therefore the mechanism for adrenalin vasodilatation in the intestine appeared to lie outside of the brain and spinal cord (fig. 1).

The adrenalin vasodilator mechanism for the hind limb is not located in the thoracic cord. This was proved in both normal and perfused limbs by destruction of the brain and cord. In the animals with the normal limb (two cats and one dog) one seemed to respond by passive dilatation while the others were active. To avoid passive effects the

hind limb was perfused after destruction of the brain and cord to the lumbar level, (one cat and one dog). Injection of adrenalin into the jugular vein caused dilatation in the perfused limb in each case.

*Lumbar cord.* The brain and spinal cord were completely pithed in two cats. Adrenalin produced dilatation in the hind limb in both. However, this seemed to be passive. The lumbar and sacral cord only were destroyed in a third cat without preventing dilatation of the hind limb from adrenalin. Where the limb effects appear to be passive a distinction can be shown by using a denervated limb. The

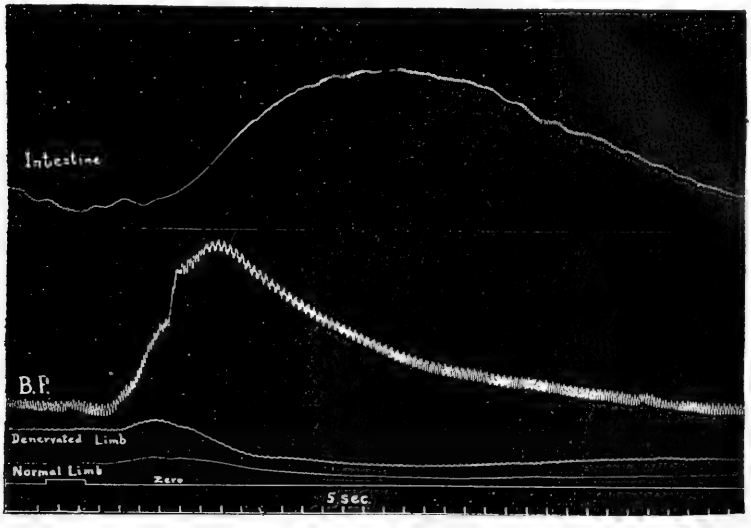


Fig. 1. The reaction of normal limb, denervated limb and intestine in a cat (weight 3 kgm.) to 3 cc., 1: 100,000 adrenalin after destruction of the brain and spinal cord. Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

latter gives earlier and more marked constriction than does the normal limb (fig. 1). We again found it necessary to resort to perfusion experiments. In addition the pithing was done with a stiff brush to insure complete destruction of the cord.

The lumbar and sacral regions of the cord were destroyed in two dogs. One hind limb was perfused and the abdominal aorta clamped. In both experiments good dilatations were obtained from injecting adrenalin into the jugular vein.

The sacral, lumbar and lower half of the thoracic cord were destroyed

in two cats. The perfused hind limb in each case dilated when adrenalin was injected into the jugular vein (fig. 2). The dose of adrenalin necessary to do this was larger than is the case in a normal limb, perhaps because of the faulty circulation in the lumbar region caused by clamping the aorta above the bifurcation. We do not find the same difference after the operation in dogs as in cats, owing no doubt partly to the great number of anastomoses in a larger animal.

These results were confirmed by experiments in which the connection between the central nervous system and the limb under observation was severed, after which no reduction was found in the dilatation caused by adrenalin. We chose dogs for this operation. The spinal

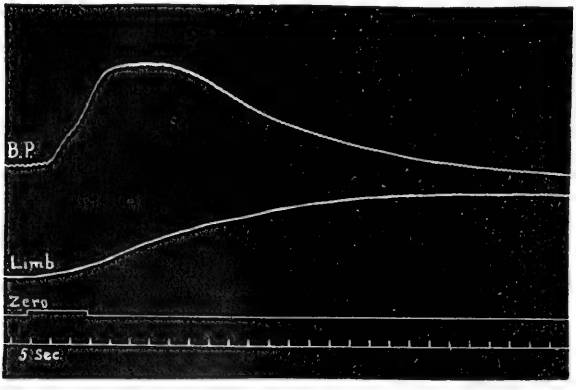


Fig. 2. Dilatation of a perfused hind limb in a cat (weight 2.5 kgm.) to 1 cc., 1: 10,000 adrenalin injected into the jugular vein after complete destruction of the spinal cord downward from the eighth thoracic level. Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

nerve roots were exposed on one side by removing the laminae and part of the transverse processes, but not the spines. Bleeding from the sinuses was stopped by hot saline packs from time to time. In the first experiment both dorsal and ventral roots of the sacral and lumbar regions were cut close to the cord on one side. The limb of that side was placed in a plethysmograph and perfused. The aorta and vena cava were tied. Injection of 0.5 cc., 1: 20,000 adrenalin into the jugular vein caused a pronounced dilatation of the perfused limb, in spite of the low blood pressure which had resulted from hemorrhage, and succeeding doses of the same strength had a similar effect. 2 cc., 1: 20,000 adrenalin caused dilatation which persisted for some time

(fig. 3). A second dog was studied in a similar manner with the same result.

We therefore came to the conclusion that the vasodilator mechanism for the hind limb could not be situated within the central nervous system but must lie either in the sympathetic or in the dorsal root ganglia or, perhaps, in both.

*Location of the mechanism for skeletal muscle.* Study of the sympathetic ganglia was next made. The right hind limb of a dog (26.0 kgm.) was placed in a plethysmograph. The last five lumbar and the

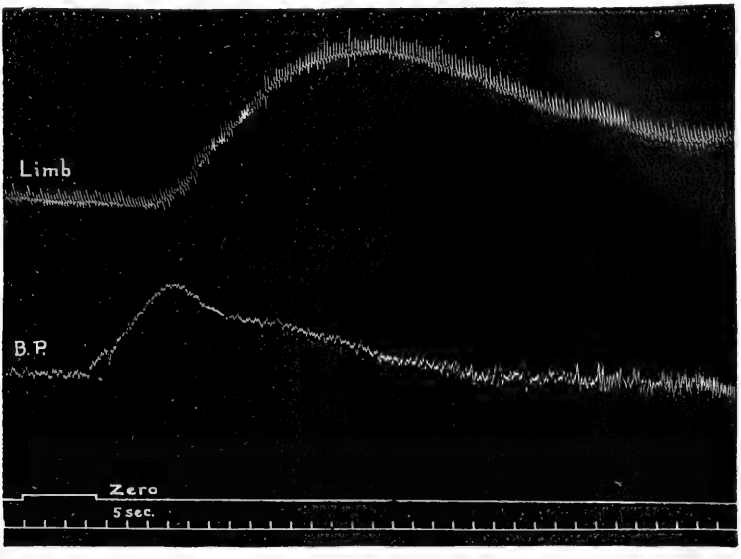


Fig. 3. Reaction of the perfused hind limb of a dog (weight 12.6 kgm.) to 2 cc., 1: 20,000 adrenalin injected into the jugular vein after cutting both dorsal and ventral roots central to the dorsal root ganglia. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

first sacral sympathetic ganglia were destroyed on the right side. The limb was next completely shut off from the circulation and perfused with warm oxygenated Ringer's solution. Injection of 1.5 cc. of 1: 20,000 adrenalin into the jugular vein caused slight dilatation of the perfused limb, while after twice the dose the dilatation was marked. A second animal was studied after destruction of the last five lumbar and the first two sacral sympathetic ganglia. The dilatation from

adrenalin was very great (fig. 4). In a third dog both sympathetic chains were completely destroyed on both sides from the third lumbar ganglion downward. Adrenalin, injected into the general circulation, caused the perfused limb to dilate as in the other experiments. The animals were always examined at the completion of the experiments to ascertain the limit of gangliar destruction.

These experiments made it appear that the limb mechanism was not in the sympathetic ganglia but in those of the dorsal roots. To determine this we approached the question in another way. The

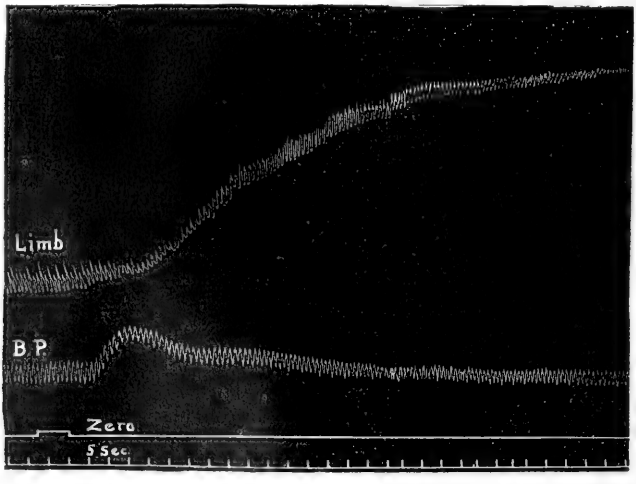


Fig. 4. Dilatation in the perfused hind limb of a dog (weight 21.6 kgm.) from the injection of 4 cc., 1: 20,000 adrenalin into the jugular vein, after removing the last five lumbar and the first two sacral sympathetic ganglia on the same side. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

dorsal and ventral roots of all lumbar and sacral nerves were cut central to the dorsal root ganglia on the right side. The right hind limb, after being placed in a plethysmograph, was completely cut off from the general circulation and immediately perfused. Adrenalin injected into the jugular vein caused dilatation of the perfused limb. The next step was removal of all dorsal root ganglia supplying the perfused limb. Following this operation injection of adrenalin into the jugular vein produced an effect on the perfused limb similar to that occurring before removal of the ganglia (see fig. 5). This was repeated in three dogs with the same result each time except that in one animal, in which

the blood pressure became quite low after removal of the dorsal root ganglia, somewhat larger doses of adrenalin were required to produce dilatation as large as before; this may perhaps be due to the poor circulation to the sympathetic ganglia because of the clamp on the abdomi-

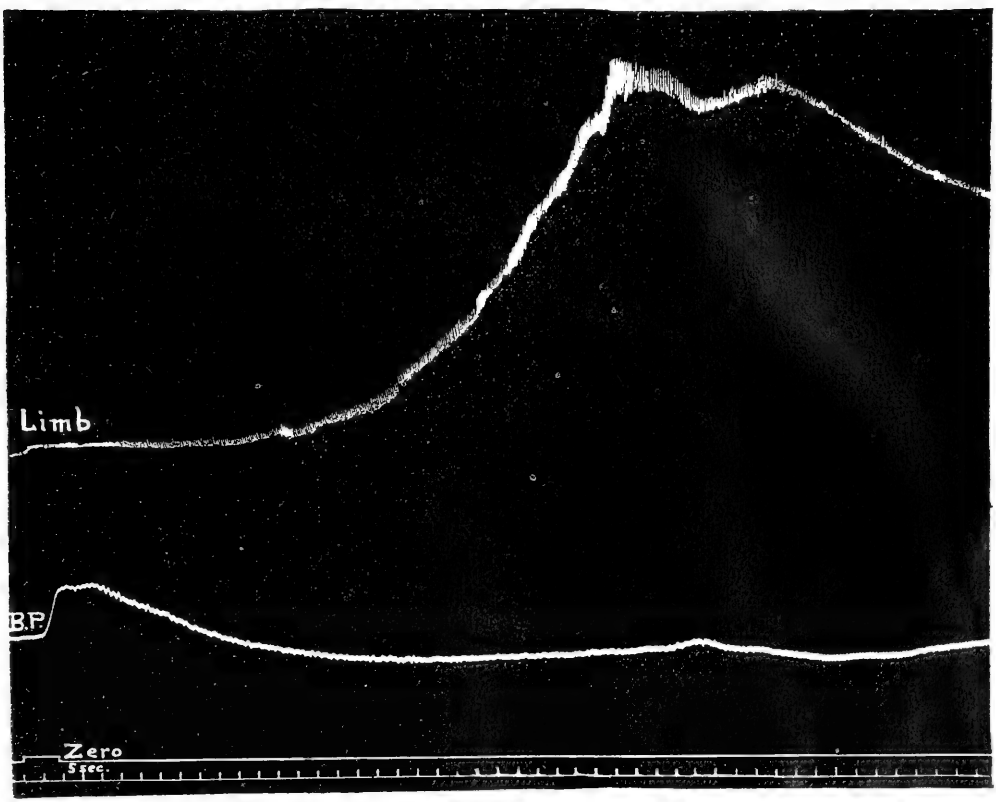


Fig. 5. Dilatation of a perfused hind limb (dog 9.0 kgm.) due to the injection of 2 cc., 1: 20,000 adrenalin into the general circulation. All dorsal root ganglia had been removed after cutting the dorsal and ventral nerve roots in the whole lumbar and sacral region on the side of the perfused limb. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

nal aorta and the general low blood pressure. We have indeed evidence of interference with the circulation of blood to the sympathetic ganglia in the delayed dilatation of the limb, i.e., in some animals the limb began to dilate long after the beginning of the change in blood pressure.

Having shown that the adrenalin vasodilator mechanism for the hind limb is in part at least to be found in the sympathetic ganglia, we next proved that the dorsal root ganglia were also in part responsible for the dilatation when adrenalin was injected. In the experiments where the sympathetic chains to the perfused limb had been completely destroyed, the remaining adrenalin vasodilator mechanisms must have been either in the dorsal root ganglia or in the spinal cord. Since from the results of our experiments on destruction of the central nervous system we were convinced that it did not contain the seat of the reaction, we wished to have definite proof that this was to be found in the dorsal root ganglia. This proof we got by destroying both abdominal and sacral sympathetic chains and cutting both dorsal and ventral roots central to the dorsal root ganglia in the whole lumbar and sacral region on the side from which the perfused limb received its supply.[1] Four dogs were studied after the above operation. In each case adrenalin injected into the general circulation caused dilatation of the perfused limb (see fig. 6). In two of the animals we then removed the dorsal root ganglia, whereupon adrenalin when injected into the general circulation failed to produce any effect upon the perfused limb.

We were able to confirm the location of the adrenalin vasodilator mechanisms for the hind limb in both sympathetic and dorsal root ganglia by the direct application of adrenalin to them.

The influence of adrenalin upon the sympathetic ganglia of the lumbar region was studied in three cats. The last two lumbar ganglia were exposed by careful dissection. A small funnel was clamped in such a position that the outlet was over one of the ganglia so that small amounts of adrenalin, poured down the funnel, bathed it. Be-

[1] We were unable to obtain satisfactory results in cats in most cases after exposure of the dorsal root ganglia, as the following experiments show. We cut dorsal nerve roots central to the ganglia in two cats on one side. In one all roots were cut from the sacral to the mid-thoracic, in the other all to the thoracic level were cut. Then the hind limb on the corresponding side was placed in a plethysmograph and perfused. In neither animal could dilatation of the perfused limb be obtained from injection of adrenalin into the general circulation. In a third cat the lumbar and sacral cord was merely exposed, after which a hind limb was placed in a plethysmograph and perfused. Even in this case no dilatation could be obtained in the perfused limb when adrenalin was injected into the general circulation, although the blood pressure was about normal (142 mm.) In the two preceding cases the blood pressure was so low that it was thought possibly a factor, (18 mm.)

tween applications the adrenalin was washed out with isotonic salt solution and taken up with absorbent cotton. The volume of the limb was recorded by means of a plethysmograph. In the first animal a 1: 100,000 solution of adrenalin produced a good dilatation of the limb with hardly any blood pressure change. A 1:10,000 solution produced marked constriction of the limb together with a steady rise in blood pressure. We consider that this constriction was not necessarily a local effect at the ganglion because of the pronounced blood pressure change which accompanied it. The second animal gave dilatation of the limb upon the first application of 1: 100,000 adrenalin, but later applications of the same concentration were without effect.

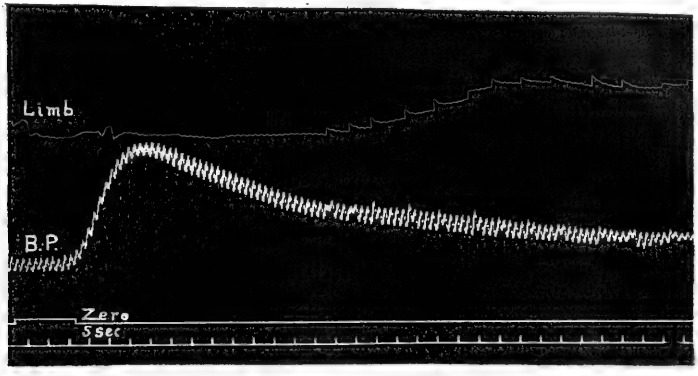


Fig. 6. Dilatation of a perfused hind limb (dog 14. kgm.) from the injection of 5.5 cc., 1: 10,000 adrenalin into the jugular vein. All sympathetic ganglia on both sides in the lumbar and sacral regions had been destroyed and all dorsal and ventral nerve roots central to the dorsal root ganglia had been cut below the thoracic level on the side of the perfused limb. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

In the third animal a 1: 100,000 solution had no effect. A 1: 10,000 solution caused a slight dilatation of the limb and a fall in blood pressure from 105 mm. to 102 mm. A 1: 5000 solution caused a more pronounced diltation of the limb and a fall in blood pressure of 25 mm. (fig. 7). A 1: 1000 solution caused marked dilatation of the limb followed later by constriction. The blood pressure change in this case was a pure fall of 21 mm.

It was more difficult to produce dilatation of the hind limb by the application of adrenalin to the dorsal root ganglia. Three dogs were studied. The lower lumbar ganglia were used, the sheaths covering

them were slit and sometimes the ganglia themselves in order to permit better access of the adrenalin. In all cases both dorsal and ventral roots were cut central to the ganglia to prevent any possible effect from the cord. The solution of adrenalin was washed away with isotonic salt solution between each application. In the first experiment, a solution of 1: 10,000 adrenalin produced a doubtful dilatation. A second dose of the same concentration produced no effect nor did stronger concentration cause any change. In a second experiment we met with greater success. Although 1: 10,000 solutions produced no effect, those of 1: 1000 caused dilatation in the limb and repeated applications of solutions of this concentration always produced the same effect. In a third case dilatation of the hind limb

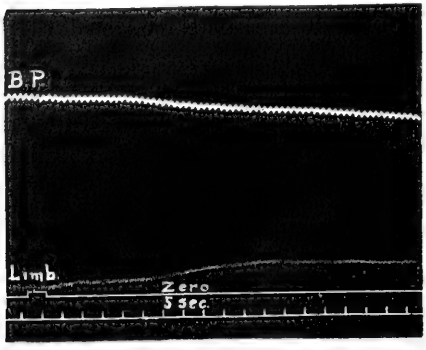


Fig. 7. Dilatation of a hind limb due to direct application of 1: 5000 adrenalin to the sixth and seventh lumbar sympathetic ganglia. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

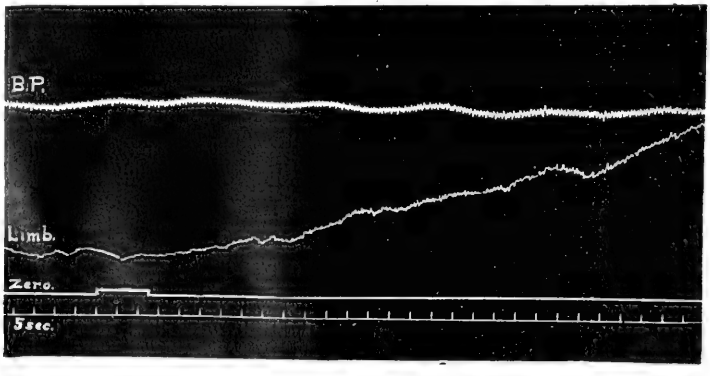


Fig. 8. Dilatation of a hind limb due to direct application of 1: 1000 adrenalin to one of the lower lumbar dorsal root ganglia. Dog, 16 kgm. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

was obtained time after time upon application of 1: 1000 adrenalin to the lower dorsal root ganglia (see fig. 8).

*Location of the intestinal mechanism.* We have previously shown (4) that the intestinal vasodilator mechanism does not function after destruction of the semilunar and superior mesenteric ganglia. At that time we stated that cutting of the splanchnic nerves produced the

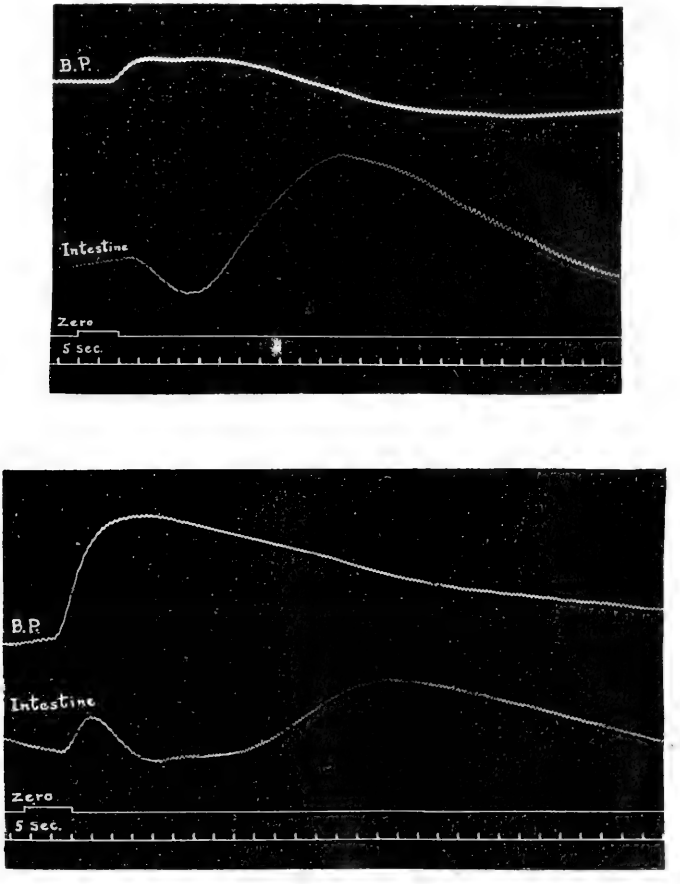


Fig. 9. Dilatation of intestine from adrenalin persists after cutting all splanchnic fibers, though it may be reduced. Upper record is the response to 0.5 cc., 1: 10,000 adrenalin before cutting the splanchnic fibers. Lower record is the response to 1 cc., 1: 10,000 adrenalin after cutting the splanchnic fibers in the same animal. (Cat weight 2.5 kgm.) Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

same result, judging from the result of the one experiment of this kind (the other experiments of the series were gangliar destruction).

It now appears from further experiments that cutting the splanchnics does not necessarily do away with the dilatation. We divided the splanchnic nerves in five cats and took records of the reaction of the intestine to adrenalin. In one the reaction was constriction only; the remaining four gave dilatation as before except that in one animal it was less marked (fig. 9) Destruction of the semilunar and superior mesenteric ganglia in one of these greatly reduced the dilatation from adrenalin but did not quite abolish it. Since, however, section of all nerves in the stalk of this loop did not prevent a small amount of dilatation, we concluded that some small part of the previous dilatation

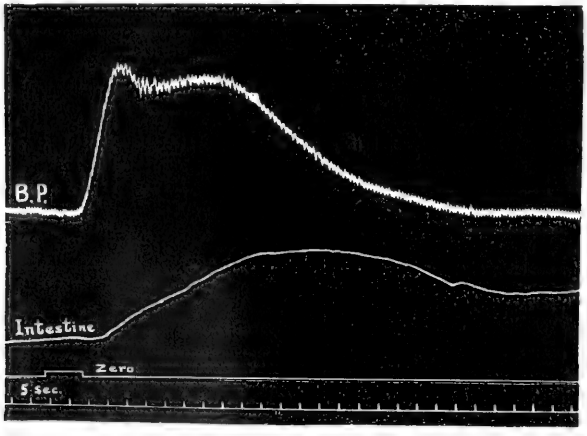


Fig. 10. Dilatation of a perfused loop of intestine of a dog (weight 13.5 kgm.) caused by the injection of 2 cc., 1: 10,000 adrenalin into the jugular vein. Postganglionic fibers intact but all central nervous connection destroyed by cutting the splanchnic fibers. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

had been either passive or else due to stimulation of peripheral structures.

In order to eliminate peripheral effects we perfused loops of intestine by the method already described (1, p. 360). All splanchnic nerve fibers and in some cases the vagi were cut. Six dogs were studied by this method. In every animal adrenalin injected into the jugular vein caused dilatation of the perfused loop of intestine, as great in amount as that usually obtained in perfused loops of which the central nervous connection was intact (fig. 10). In two dogs the dilatation was often preceded by constriction. Cutting the nerves in the stalk of the perfused loop did away with all effects of the injection. Isola-

tion of the collateral ganglia from the central nervous system was verified in each instance by post-mortem dissection. Two cases showed incomplete section of the lesser splanchnics. In the remaining four the destruction of central nervous connection with the ganglia was found to be complete.

If sympathetic ganglia control the adrenalin vasodilatation in the intestine, it is natural to suppose that suitable doses of nicotine should reduce the dilatation by paralyzing the sympathetic nerve cells. This was found to be the case. A cat (3.2 kgm.) was given intravenously a total of 2.1 cc. of a 0.1 per cent nicotine solution divided into four doses. The dilatation in the intestine from adrenalin was smaller in amount after nicotine than before. A second cat (2.6 kgm.) gave a similar result after an intravenous dose of nicotine (1.7 cc. of a 0.1 per cent solution). The intravenous injection in a third animal prevented the intestinal dilatation altogether (fig. 11). In a fourth cat dilatation was prevented by painting the superior mesenteric ganglion with a 1 per cent nicotine solution.

Vasodilatation of the intestine, therefore, is apparently caused by the action of the adrenalin upon some structure in the superior mesenteric ganglion. As an added proof of this we have been able to cause dilatation of the intestine by the direct application of adrenalin solution to the superior mesenteric ganglion. The intestine of a cat was placed in an oncometer. The mesentery was cut and separated from the superior mesenteric ganglion in such a way that a pocket could be made by engaging the cut surface of the mesentery with haemostats, to form a pool of the solution of adrenalin around the ganglion. The solution was simply poured into the pocket and between each application it was washed away with normal saline solution, which was afterwards removed by sponging. The following results were obtained: A small dilatation of the intestine was produced by a 1: 20,000 solution, ten minutes later a 1: 5000 solution produced a more marked dilatation and finally a 1: 1000 solution produced a dilatation which continued to increase over a longer period than the preceding, although the first effects were about the same (fig. 12). In no case was there any appreciable effect upon the blood pressure.

In view of the evidence advanced above, that the dorsal root ganglia contain an adrenalin vasodilator mechanism for the hind limb, it was thought possible that there might be a similar one for the intestine and that this might respond to direct application of a solution of adrenalin. With the results of Bayliss (5) in mind we judged that the

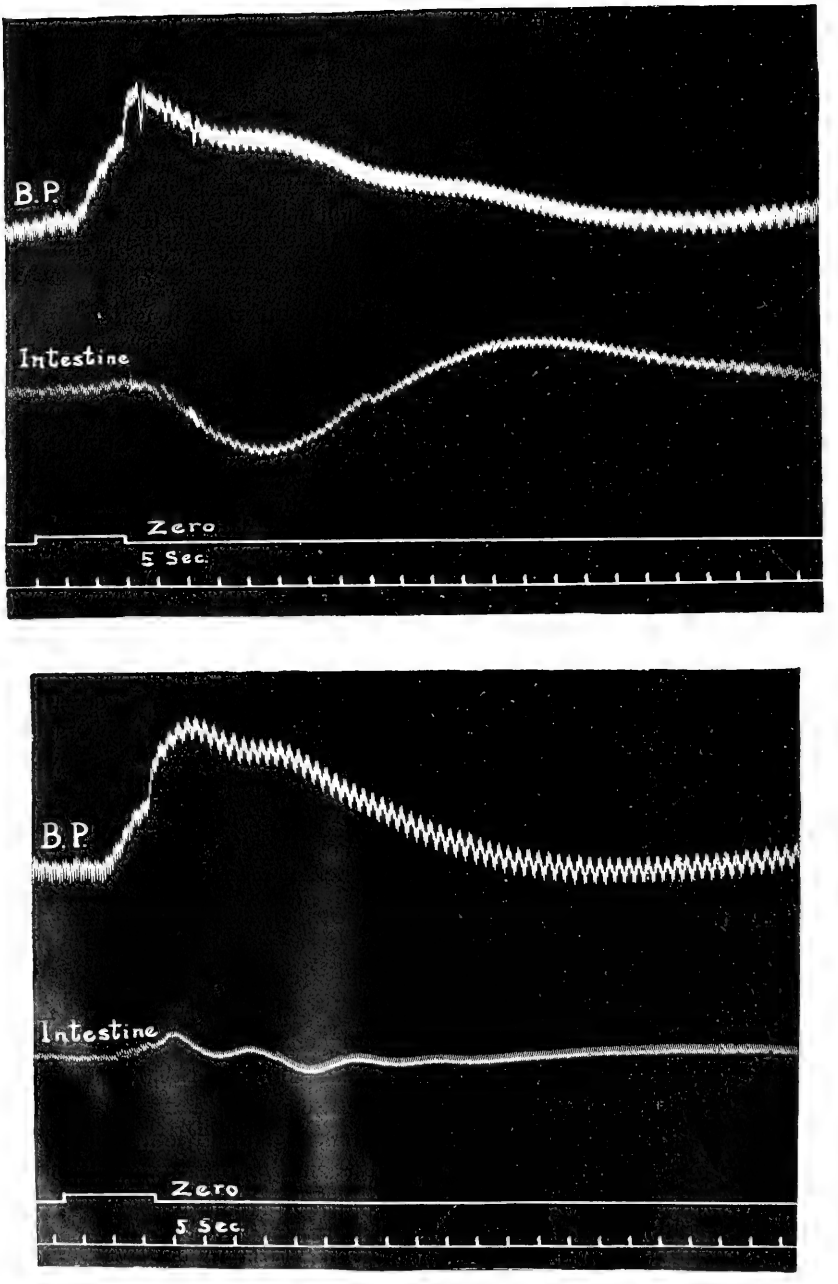


Fig. 11. Failure of intestinal dilatation from adrenalin due to paralysis of the mechanism by nicotine. Upper record, response to 2 cc., 1: 100,000 adrenalin before nicotine. Lower record, response to 2 cc., 1: 100,000 adrenalin after injection of nicotine in the same animal (cat, weight 2.3 kgm.) Base of bellows 20 mm. x 21 mm. (Reduced one-fourth.)

dorsal root ganglia most likely to cause this reaction were those of the twelfth and thirteenth thoracic nerves. We have been able to show that such a mechanism exists, although several of our experi-

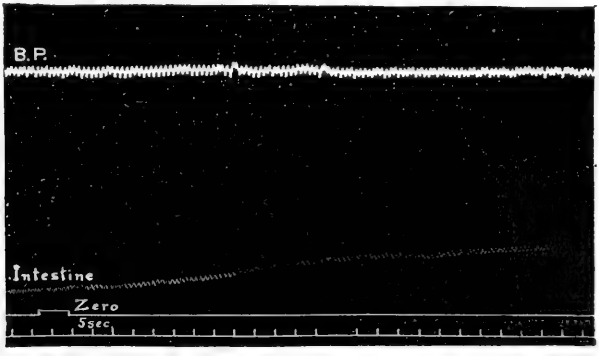


Fig. 12. Dilatation of the intestine due to direct application of 1: 1000 adrenalin to the superior mesenteric ganglion. Cat. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

ments gave negative results. We investigated seven animals in all, three dogs and four cats, taking records of the volume changes of a loop of intestine on application of a solution of adrenalin 1: 1000 to the ganglia. The preparation of these in the dog was like that described

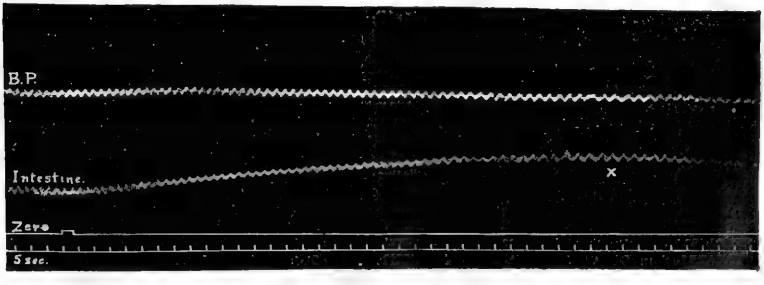


Fig. 13. Dilatation of the intestine caused by application of 1: 1000 adrenalin to a split dorsal root ganglion. Adrenalin washed away with isotonic salt solution at *X*. Cat. Base of bellows 10 mm. x 19 mm. (Reduced three-fifths.)

above. In the cat we cut the cord across, drew the cut ends back and got access to the ganglia from their central ends, thus avoiding excessive bleeding. In all cases we found it necessary to cut the ganglia

longitudinally to allow the solution free access to the cells. Of the dogs, one showed no change and the two others only slight dilatations, and these not always occurring. In one case constriction took place. Two of the experiments on cats were more successful. In both the application to the ganglia of three or four drops of the adrenalin solution caused a gradual dilatation of the intestine, which was accompanied by no change or by a slight fall in blood pressure and which gradually disappeared after the ganglia had been washed with saline (fig. 13).

## DISCUSSION

It is not surprising to find that the central nervous system does not contain the structures stimulated by adrenalin in bringing about vasodilatation. Cannon and Lyman (6) obtained a fall in blood pressure from adrenalin after total destruction of the central nervous system, if ergotoxine had previously been given. Of course it cannot definitely be said that the central nervous system has nothing to do with the problem, since destruction of portions of the brain or cord inhibits or modifies the response, as is evident in the reversal of blood pressure effects. What our results go to show is that the main seat of the reaction is in the sympathetic and dorsal root ganglia. The same conclusion has been arrived at by widely different ways, viz., 1, by perfusion of the organ, together with destruction or removal of the central nervous system and of one or the other set of ganglia, which might be the seat of the reaction; 2, by the destruction of the ganglia in question, thus preventing the dilatation; 3, by the direct application of adrenalin to the ganglia. The fact that to these ganglia is due the greater part of the dilatation caused by adrenalin does not exclude the possibility of some peripheral action on the dilator nerve endings, as various investigators have suggested, notably Gruber in a recent paper (7). Further research on this question is in progress in this laboratory. At present we are not in a position to say whether the gangliar or the peripheral action is more effective in bringing about dilatation.

The nature of the action of adrenalin on the cells of the sympathetic ganglia is still uncertain, whether it is an inhibition of the constrictor elements or a stimulation of a dilator. The first, in the light of the stimulating action of this hormone on the endings of the fibers from these cells seems improbable. In spite of the negative experiments of Bayliss (5) and others we are inclined to attribute our results to a

stimulation of vasodilator cells. If the existence of such cells is a fact, the part, whether cell or synapse, which adrenalin affects, is still uncertain. That it cannot stimulate the fiber directly is evident because no dilatation has ever been obtained in a perfused organ, the ganglia to which have been removed, no matter how few minutes before.

The nature of the adrenalin vasodilator mechanism of the dorsal root ganglia is uncertain, nor is there any evidence as to whether it is similar to that in the sympathetic ganglia. Dogiel (8) has described so-called sympathetic cells in the dorsal root ganglia, but little seems to be known concerning them. Whatever the structure may be, the impulses which are started must be antidromic. From this arises the question of the possible identity of these impulses with those described by Bayliss (9), which brought about dilatation by their action on the vessels of the skin. We have not been able to show conclusively that the dilatation which takes place in a perfused limb, all the nervous connections of which have been destroyed except those with the dorsal root ganglia, is not caused by the vessels of the skin, but all the evidence tends to make it improbable.

## SUMMARY

1. Dilatation of the hind limb is brought about by the action of adrenalin on structures located in the sympathetic ganglia of the lower lumbar and sacral regions and in the dorsal root ganglia of the nerves supplying the limb.

2. Dilatation of the intestine is brought about by the action of adrenalin on structures in the superior mesenteric ganglion and in the dorsal root ganglia of the lower thoracic region.

3. Our results tend to support the view that the sympathetic system contains vasodilator fibers to the intestine and to the hind limb.

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# VASCULAR CHANGES PRODUCED BY ADRENALIN IN VERTEBRATES

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The majority of physiologists still teach that adrenalin is essentially constrictor in its effect upon the blood vessels, ignoring the fact that doses which are probably physiological in their magnitude cause dilatation in a large proportion of vessels. These teachings are founded upon the older experiments in which massive doses of the hormone were used. Such amounts of adrenalin are probably never secreted by the adrenal glands (1, 2, 3). Although in the last few years it has been conclusively proven that small quantities of adrenalin cause vasodilatation and a fall in blood pressure as a result (4, 5, 6, 10) the fact is still ignored. This situation may be easily explained, for, among the common laboratory mammals some give evidence of vasodilatation while others consistently fail to do so. These animals which have been found to give positive proof of dilatation belong to the carnivores, while those that do not belong to the rodents. In face of the experimental facts it was as easy to believe the response of cats and dogs exceptional, as that the effect in rabbits was different from that in other animals. In view of this disagreement, it was perfectly natural to assume that the action of adrenalin in cats and dogs was unusual, since it did not conform to other beliefs such as the absence of vasodilator fibers in the sympathetic nervous system.

This research was undertaken with the object of determining whether the dilator action of adrenalin was confined to the carnivores. It was conceivable that other groups might give a similar action, although none were known to do so; accordingly a survey was made of all the groups available. The results have been sufficient to remove all doubt as to the general occurrence of vasodilatation from adrenalin.

A brief sketch of our present knowledge concerning this dilatation is needed as a foundation for this research. The nature of the mechanism on which adrenalin acts was worked out largely by experiments upon cats and dogs. Those experiments have proven that a differential effect is produced—dilatation in skeletal muscle (5, 6) and intestine, (large doses)—constriction in skin (6), intestine (small doses), kidney (8, 10), bone (16), thyroid (15) and spleen (7, 10). With small doses, the vessels in skeletal muscle more than counteract the constriction in the skin and abdominal viscera, so that a fall in blood pressure results. When the amount of adrenalin is sufficiently large, the constriction of skin and visceral vessels (excepting intestine) becomes great enough to more than compensate for the dilatation in skeletal muscle, thus producing a rise in blood pressure.

The dilatation produced by adrenalin has been shown to be brought about by dilator mechanisms located in the sympathetic and dorsal root ganglia (12) as well as in a "terminal" receptive substance which has been called the myoneural junction (13, 14). The latter, a counterpart of the constrictor myoneural junction, is assumed to be associated with dilator fibers.

## METHODS

The methods employed in this research were those already described in work from this laboratory (10, 11, 12).

All animals, unless otherwise stated, were anaesthetized with ether. Blood pressure was taken from the carotid artery, except in the fowl, in which case the sciatic artery was used. Injections were made into the jugular vein.

Solutions of adrenalin chloride were made up by diluting the 1:1,000 preparation of Parke, Davis & Co. Volume changes were registered by means of Brodie's bellows. The plethysmograph for the limb was either of the type which enclosed the paw, or else like a cuff, so that the paw might be excluded (13). It was necessary to use artificial respiration in the fowl when the abdomen was opened.

## RESULTS

### **Reptilia** (Chelydra)

A snapping turtle (5.3 kgm.) was employed as representative of the reptiles. Doses of adrenalin as small as 0.2 c.c., 1:1,000,000 were tried with no effect upon the blood pressure. Even 0.5 c.c., 1:100,000 had no effect. 1.0 c.c., of the latter concentration caused a rise from 46 mm. to 50 mm. 0.4 c.c. 1:10,000 caused a change from 44 to 54 mm. 1.0 c.c. of the same solution produced about the same effect. Indeed it was found that with large doses, sensitiveness to adrenalin was soon lost. 0.5 c.c., 1:1,000 following the above, increased the pressure only 6 mm. from 51 mm. Repetition of this had no effect, nor did twice the dose. Two months later, the blood pressure and intestinal effects were studied in the same animal. The blood pressure responses were similar. The in-

testine always gave constriction when there was any effect. This was observed with doses ranging from 0.5 c.c., 1:100,000 to 3.0 c.c., 1:10,000. After the latter dose, 1.0 c.c., 1:1,000 produced no intestinal change.

Although only tentative conclusions can be drawn from a single animal, they are at least valuable when considered in connection with other vertebrates low in the scale. We have found that the vascular system of the turtle is not very sensitive to adrenalin and that there is evidence of only a constrictor mechanism. The failure to obtain a fall in blood pressure or a dilatation of the intestine indicate an absence of the dilator mechanisms.

**Aves** (Gallus)

The fowl serves as an example of the warm blooded vertebrate other than the mammal. It is much more sensitive to adrenalin than are the cold-blooded vertebrates. Moreover it does not easily lose its power to respond to this hormone, even after numerous doses.

Constriction is the only effect produced by adrenalin in the fowl. Both the limb (Fig. 1) and the intes-

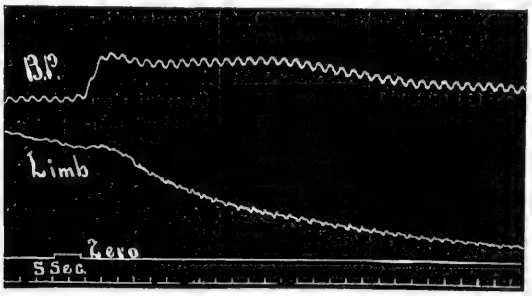


FIG. 1
Effect of 0.5 c.c., 1:100,000 adrenalin upon the limb in the fowl, 1.0 kgm. (Reduced ⅔.)

tine (Fig. 2) respond in this way. From a study of

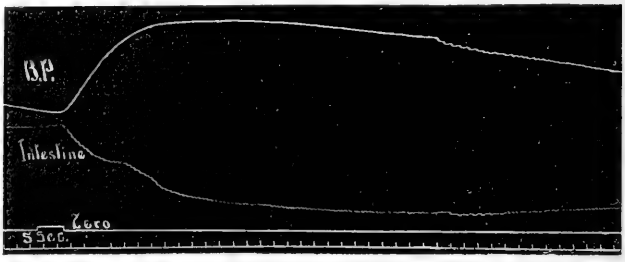


FIG. 2
Prolonged constriction of the intestine in the fowl. (0.92 kgm.) Produced by 0.5 c.c., 1:10,000 adrenalin. (Reduced ⅔).

seven animals no evidence of the existence of the adrenalin vasodilator mechanisms (Table 1) has been found.

TABLE I.
RESPONSE TO ADRENALIN IN THE FOWL

| Weight in kgm. | Dose | Blood pressure change in mm. of mercury | Limb | Intestine |
|---|---|---|---|---|
| 1.1 | 0.2 cc 1:1,000,000 | | Slight constriction | |
| | 0.5 cc 1:100,000 | 109-138 | Constriction | |
| | 1.0 cc " | 114-182 | Marked constriction | |
| 1.0 | 0.1 cc 1:100,000 | 118-130 | Constriction | |
| | 0.5 cc " | 106-134 | Marked constriction | |
| 0.92 | 0.2 cc " | 65- 79 | Constriction | Constriction |
| | 0.5 cc " | 95-175 | | Marked constriction |
| 0.85 | 0.2 cc 1:1,000,000 | 52- 54 | Constriction | |
| | 0.5 cc 1:100,000 | 55- 65 | Marked constriction | Constriction |
| | 1.0 cc " | 80-119 | | Constriction |
| 0.95 | 0.5 cc 1:100,000 | | | Constriction |
| | 0.5 cc 1:10,000 | | | Very marked constriction |

## MAMMALIA

**Marsupialia** (Didelphys)

A single opossum about two-thirds grown (weight 1.3 kgm.) was used in this research. A fall in blood

pressure (Fig. 3) was easily obtained from adrenalin.

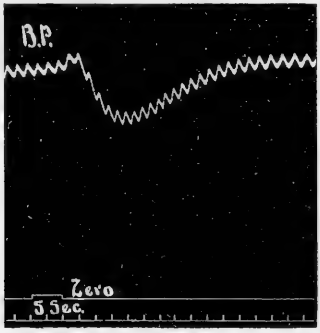


FIG. 3
Blood pressure fall in the opossum produced by 0.2 c.c., 1:100,000 adrenalin. (Reduced ⅜.)

This was usually preceded by a brief rise. With larger doses pure pressor effects resulted.

Although the limb included in the plethysmograph possessed a smaller proportion of muscle than that in most mammals it gave active dilatation (Fig. 4) ex-

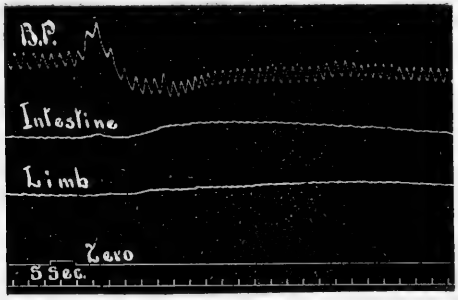


FIG. 4
Dilatation of limb and intestine in the opossum caused by a depressor dose of adrenalin, 0.2 c.c., 1:10,000. (Reduced ⅜.)

cept when large doses were used. The intestine dilated actively in response to adrenalin (Fig. 5), the dilatation becoming very marked with large doses (Table II).

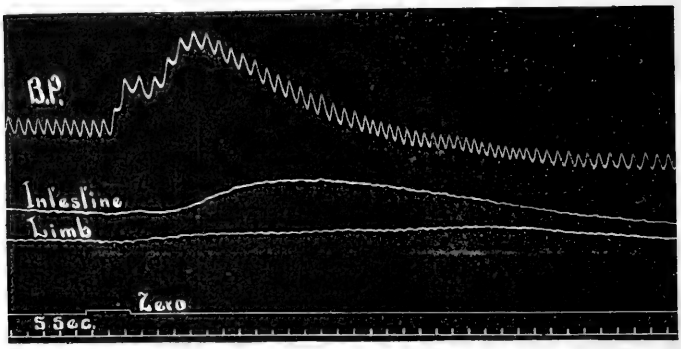


FIG. 5
Marked dilatation of the intestine in the opossum resulting from the injection of a pressor dose of adrenalin 0.5 c.c., 1:10,000. (Reduced ⅗.)

TABLE II.
RESPONSE OF THE OPOSSUM TO ADRENALIN

| Dose | Blood pressure change in mm. of mercury | Response of limb | Response of Intestine |
|---|---|---|---|
| 0.05 cc 1:100,000 | 140-142-136 | | Dilatation |
| 0.1 cc " | 138-144-128 | | Dilatation |
| 0.2 cc " | 144-151-135 | Slight constriction | Dilatation |
| 0.5 cc " | 118-125-106 | Dilatation | Constriction and dilatation |
| 0.5 cc 1:10,000 | 123-180 | Small dilatation | Marked dilatation |
| 1.0 cc " | 98-215 | Small dilatation | Marked dilatation |

We may conclude then that the opossum and probably all marsupials possess adrenalin vasodilator mechanisms similar to those in the cat and dog.

## UNGULATA

### Perissodactyla (Equus)

Unfortunately the horse which we used was in such poor condition that it cannot be considered typical. It was anaesthetized with chloroform, and 1:1000 adrenalin was injected in every instance. In no case was there a fall in blood pressure. 5.0 c.c., adrenalin changed the pressure from 114 mm. to 162 mm.

10.0 c.c. increased the pressure from 80 to 260 mm.

Attempts to produce dilatation of the intestine were successful when 25.0 c.c. was injected, there being a strong constriction followed by a dilatation. 20.0 c.c. produced constriction only.

Intestinal dilatation was the only indication of the presence of an adrenalin vasodilator mechanism in the horse.

**Artiodactyla** (Capra)

In view of the unsatisfactory condition of the horse, it was imperative that another animal belonging to the ungulates be tried. An experiment with a goat (weight 13.0 kgm.) removed all doubt as to the existence of adrenalin vasodilator mechanisms in this order. A depressor effect (Fig. 6) as well as

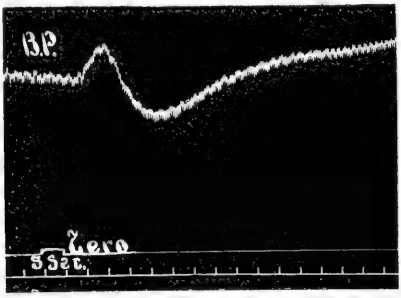


FIG. 6
Fall in blood pressure from 0.4 c.c., 1:100,000 adrenalin in the goat, 13.0 kgm. (Reduced ½.)

active dilatation of the limb (Fig. 7) could be obtained from the injection of small amounts of adrenalin. However nothing but constriction in the intestine (Fig. 8) resulted from even large doses of adrenalin until perfusion was attempted. A loop of intestine, with nerves intact, but shut off from the general circulation and perfused with oxygenated Ringer's solution gave pronounced dilatation both when

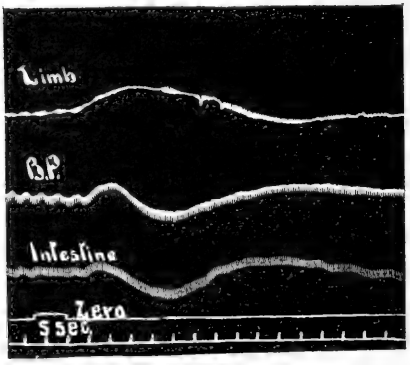


FIG. 7
Dilatation of the limb and constriction of the intestine produced by 0.5 c.c., 1:100,000 adrenalin in the goat. (Reduced ½.)

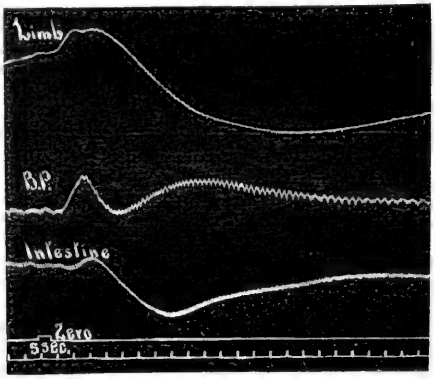


FIG. 8
Constriction in the limb and intestine caused by 1.0 c.c., 1:10,000 adrenalin, goat. (Reduced ⅗.)

TABLE III.
RESPONSE OF THE GOAT TO ADRENALIN

| Dose | Blood pressure, mm. of mercury | Change in Limb | Change in Intestine |
|---|---|---|---|
| 0.4 cc 1:100,000 | 110-128-94 | Dilatation | |
| 0.5 cc " | 62- 68-53 | Dilatation | Constriction |
| 0.7 cc " | 72- 80-61 | Dilatation and Constriction | Constriction |
| 0.3 cc 1:20,000 | 80- 92-66 | Constriction | Constriction |
| 1.0 cc " | 84-106 | Constriction | Constriction |
| 1.0 cc " | 28- 26 | | Dilatation* |
| 2.0 cc " | | | Dilatation* |

*Intestine perfused.

1.0 c.c., and when 2.0 c.c., 1:20,000 adrenalin were injected into the jugular vein. (Table III).

Our experiments thus indicate that the mechanisms for dilatation from adrenalin are found in the ungulates.

## CARNIVORA

Cats and dogs were the only Mammals known to possess adrenalin vasodilator mechanisms before this research was undertaken. We were interested in finding out whether all families in this order reacted to adrenalin in the same way. Two other families were therefore investigated, viz.—the *mustelidae* and the *procyonidae*.

### Mustelidæ (Putorius)

Study of an old ferret (weight 0.6 kgm.) indicated the presence of the vasodilator mechanisms. This evidence was largely limited to depressor effects of adrenalin, 0.2 c.c., 1:1,000,000 causing a fall of 6 mm. from 152 mm. In one instance dilatation of the limb was obtained.

### Procyonidæ (Procyon)

Typical adrenalin vasodilator effects were obtained in the raccoon. A marked fall in blood pressure was produced by small doses. (Fig. 9). Dilatation of

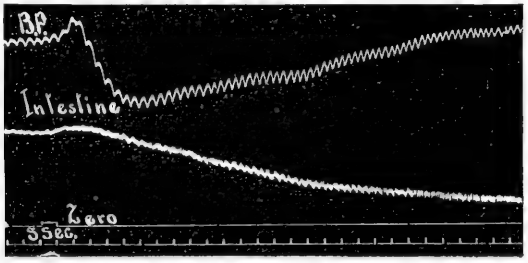


FIG. 9
Fall in blood pressure and constriction of the intestine produced by the injection of 0.2 c.c., 1:100,000 adrenalin. Raccoon. (Reduced ⅜.)

the limb sometimes resulted from depressor doses. Constriction (Fig. 9) or constriction and dilatation (Fig. 10) occurred in the intestine depending upon

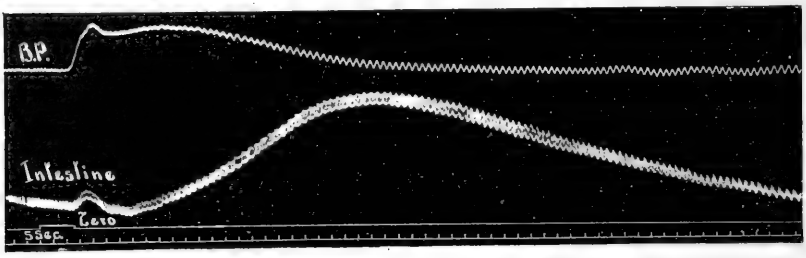


FIG. 10
Dilatation of the intestine produced by 0.3 c.c., 1:10,000 adrenalin, raccoon. (Reduced ⅔.)

the amount of adrenalin injected, just as in cats and dogs.

## RODENTIA

A reason already given that the cat and the dog have been considered possibly exceptions in their behavior toward adrenalin is the fact that the rabbit does not give the same results. We will show, however, judging from the rat and rabbit that rodents are an exception in their behavior toward adrenalin and that the reaction of the cat and dog is the typical one for most mammals.

### **Muridæ** (Mus)

A fall in blood pressure could not be obtained in the white rat. In an animal weighing 0.23 kgm., 0.05 c.c., 1:100,000 adrenalin caused a pure rise from 69 to 83 mm. Smaller doses such as 0.3 c.c., 1:1,000,000 had no effect.

### **Leporidæ** (Lepus)

We have never obtained evidence of the presence of adrenalin vasodilator mechanisms in the rabbit. At least twelve rabbits have been examined in this

connection. It has always been our experience that whenever a dose of adrenalin is large enough to produce any effect, nothing put a pure rise of blood pressure results.

There might, however, be a differential effect without a fall in blood pressure. In one experiment the coeliac, superior mesenteric, inferior mesenteric and renal arteries were tied (5) without changing the reaction to adrenalin.

The limb reaction was determined in four animals. With small doses a dilatation which appeared to be passive, sometimes occurred. When the amount of adrenalin was increased constriction was produced. (Fig. 11). The presence of active dilatation was

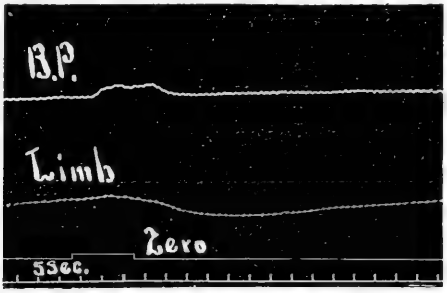


FIG. 11
Constriction of the hind limb of a rabbit (2.4 kgm.) from 0.4 c.c., 1:100,000 adrenalin. (Reduced ½.)

then sought in another way. The hind limb of an animal was perfused with Ringer's solution, and adrenalin was injected into the jugular vein. If dilator mechanisms sensitive to adrenalin, exist in the sympathetic and dorsal root ganglia, the limb under these conditions should respond. The injection of even large doses of adrenalin into the jugular vein was without effect. Injection into the perfusion fluid as it entered the iliac artery was also without

effect in one animal, while in a second rabbit the first two doses (0.5 c.c. and 0.1 c.c., 1:100,000) caused constriction, but later doses had no effect.

In one experiment the sciatic and femoral nerves in one limb had been cut seventeen days before. However no evidence of a terminal dilator mechanism could be obtained. In the cat and dog this has been obtained easily by such a method (13, 14).

Very small passive dilatations were produced in the denervated limb of the rabbit when adrenalin was injected into the general circulation. This limb was perfused later, doses of adrenalin varying from 1:1,000,000 to 1:10,000 concentration being injected into the fluid, but without result.

The reaction of the intestine was observed in four animals. In one, there was small passive dilatation with small doses. The other three constricted with doses of this size. In all there was prolonged constriction with large doses (Fig. 12). As an illustra-

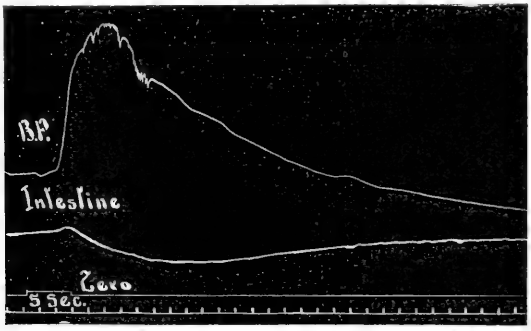


FIG. 12
Constriction of the intestine of a rabbit due to the injection 0.5 c.c., 1:10,000 adrenalin. (Reduced ⅔.)

tion of the amount of constriction; a loop 37 c.c. in volume constricted .72 c.c. after the injection of 0.5 c.c., 1:10,000 into the general circulation.

In an unpublished research we have found that there is a dilator mechanism for the kidney located in the aortico-renal ganglion. One of the methods employed has been to apply adrenalin solutions to the ganglion, noting the volume change in the kidney. We did this in a rabbit, but obtained constriction in the kidney instead of dilatation.

We conclude from our results that rodents do not possess adrenalin vasodilator mechanisms.

## PRIMATES

### **Monkey** (Pithecus)

Adrenalin vasodilator mechanisms are present in the monkey (Table IV). Excellent dilatations of a

TABLE IV.
RESPONSE OF THE MONKEY TO ADRENALIN

| Dose | Blood pressure change in mm. of Mercury | Limb | Intestine |
|---|---|---|---|
| 0.1 cc 1:100,000 | 96-101-92 | Dilatation | Constriction and slight dilatation |
| 0.4 " " | 94- 98-86 | Dilatation | |
| 1.0 " " | 80- 98 | Very marked dilatation | |
| 0.3 " 1:10,000 | 86-124 | Marked dilatation | Marked constriction and dilatation |
| 1.3 " " | 64-166 | Marked dilatation | Marked constriction and dilatation |
| 2.5 " " | 64-177 | Marked dilatation and constriction | Marked constriction and dilatation |

leg were produced (Figs. 14 and 15) by doses of adrenalin ranging from 0.4 c.c., 1:100,000 to 0.7 c.c., 1:10,000 (weight of animal 5.2 kgm.) (The foot was not included in the plethysmograph). Indeed, a large dose of adrenalin was required to cause reversal in the limb (Fig. 16). By perfusing the limb and injecting adrenalin into the jugular vein we attempted to bring the gangliar mechanism into action, without

result. We thought this might be due to failure of the adrenalin to reach the ganglia on account of

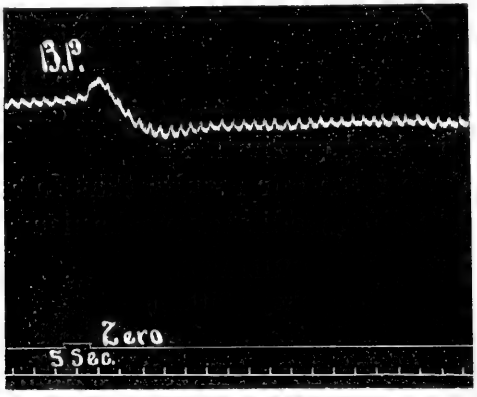


FIG. 13
Fall in blood pressure in the monkey (5.2 kgm.) resulting from 0.4 c.c., 1:100,000 adrenalin. (Reduced ½.)

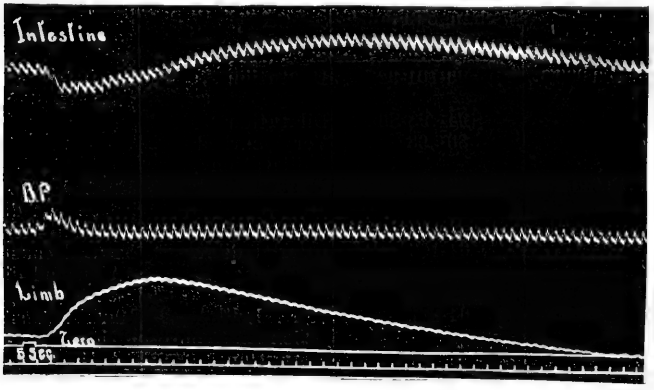


FIG. 14
Response to 0.5 c.c., 1:100,000 adrenalin in the monkey. Constriction followed by dilatation of the intestine. Marked dilatation of the limb. (Reduced ⅔.)

clamping the aorta too high up, as that has frequently been the case in cats. On the other hand injection of the hormone into the perfusion fluid easily produced dilatation. The explanation, therefore, might be that the vasodilator myoneural junction and not the gan-

gliar mechanism was the source of the dilatation. We are inclined to doubt this as being typical, for there is no reason to believe that the monkey is different from the cat and dog in which the gangliar mechanism is an important source of adrenalin vasodilatation (13).

The intestinal mechanism in the monkey worked

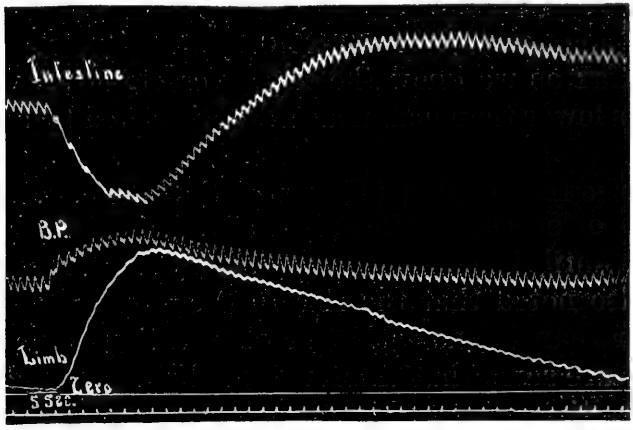


FIG. 15
Effect of a larger dose of adrenalin, 0.3 c.c., 1:10,000 in the monkey. (Reduced ⅔.)

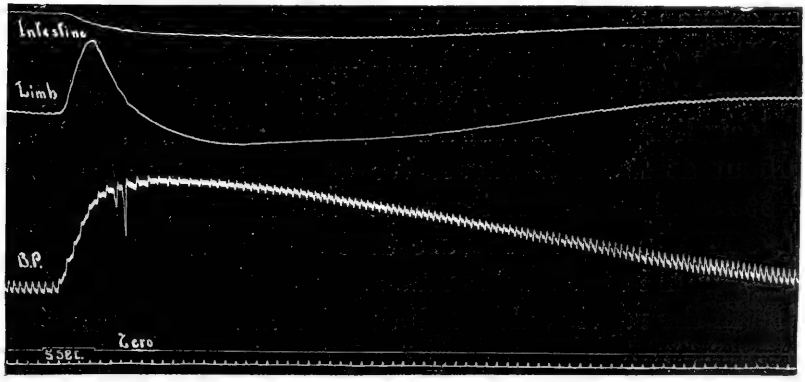


FIG. 16
Reversal in the limb produced by a large dose of adrenalin, 2.5 c.c., 1:10,000 in the monkey. (Reduced ¾.)

very well (Figs. 14 and 15) until large doses of adrenalin were used when constriction only was obtained (Fig. 16).

A fall in blood pressure was obtained from the injection of small doses of adrenalin (Fig. 13), but as sometimes happens in cats or dogs the fall became small or almost disappeared after a few doses had been injected (Fig. 14).

## DISCUSSION

So far as we know the blood vessels of all vertebrates lower than mammals are constricted by adrenalin.

In the frog Burket (17) found that the constrictor effect of succeeding doses of adrenalin rapidly declines until there remains only a very small response. He also noted that the rise in pressure lasts much longer than in the cat. Our observations upon the turtle are somewhat similar, there being a rapid loss in sensitiveness to adrenalin and a prolonged effect when the rise is produced. In addition attention should be called to the fact that the threshold for a blood pressure response is much higher in the reptiles than in the mammals.

It is of interest to note also that birds resemble mammals in some respects in their behavior toward adrenalin. The threshold for adrenalin response is about as low and successive doses of adrenalin do not readily decrease the sensitiveness. The percentage rise in blood pressure that can be produced by adrenalin in the fowl is much greater than that possible in the reptile, although the rise in blood pressure is more prolonged in the fowl than in the mammal. It may be partly due to the absence of dilator mechanisms which could be affected by adrenalin and thus

tend to offset the constrictor effect. Dale (22) was able partially to paralyze the constrictor mechanism in the fowl, but he obtained no fall in blood pressure from adrenalin.

Besides the carnivores and rodents which have been extensively studied by different investigators, a few observations have been made upon the ungulates and primates. Barger and Dale (18) paralyzed the vaso-constrictor mechanism in the pig and the goat with ergotoxin, but failed to obtain a fall in blood pressure when adrenalin was injected. Barbour and Prince (19), in experiments with perfused hearts obtained dilatation of coronary vessels in the ox, sheep, pig, and rabbit, but constriction in the monkey.

Auer and Meltzer (20) obtained usually a rise, but sometimes also a fall in blood pressure from the intraspinal injection of large amounts of adrenalin in the monkey.

We have been able to show in all orders of mammals which we have studied, except the rodents, that both adrenalin vasodilator mechanisms (for skeletal muscle and intestine) are present. On the other hand, we have been unable to prove the presence of such mechanisms in the rodents. Moreover, no one else (21, 23) has ever been able to produce a fall in blood pressure by the injection of adrenalin in the rabbit. Dale (22) was unable to obtain a reversel by the use of ergot, although he abolished the pressor effect of adrenalin.

Dilatation from adrenalin had been observed in the rabbit. The Meltzers (24) obtained dilatation of the ear vessels of the rabbit from the subcutaneous injection of adrenalin. Ogawa (25) produced dilatation

of the perfused kidney, intestine and hind limbs of the rabbit by adrenalin. However, he usually obtained constriction of the kidney even with dilute solutions. He did not secure a primary dilatation in the limb. We are led to conclude as a result of our experiments that even though adrenalin vasodilatation may occur in the rabbit it is relatively unimportant.

In conclusion, we are justified in assuming that the usual vasomotor reaction in skeletal muscle is dilatation with moderate doses of adrenalin, rodents being exceptional; and because of the uniform occurrence in other mammalian orders as well as the presence in the monkey we have considerable reason for believing that these mechanisms are also present in man.

We wish to thank Lois McPhedran Fraser for assistance in a part of this research.

## SUMMARY

1. Birds and reptiles possess no adrenalin vasodilator mechanisms.
2. A small amount of adrenalin produces a fall in blood pressure in marsupials, ungulates, carnivores and primates.
3. Adrenalin vasodilator mechanisms for the limb and intestine are present in marsupials, ungulates, carnivores and primates.
4. Rodents are exceptional in their reaction to adrenalin, vasodilator mechanisms sensitive to this hormone being absent.
5. Dilatation in the blood vessels of skeletal muscle is the usual response to adrenalin in mammals.

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## PHYSIOLOGICAL SERIES

No. 19: SIMPLIFIED GAS ANALYSIS, BY PROFESSOR J. J. R. MACLEOD

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# SIMPLIFIED GAS ANALYSIS

By J. J. R. Macleod, M.B., Toronto, Canada

## NO. II. BURETTE WITHOUT STOPCOCKS FOR GAS ANALYSIS

FOR purposes of teaching, as well as for occasional use in clinical practice, the usual types of gas burette are unsuitable because of the care and attention that has to be given them in order to prevent "freezing" of the stopcocks. Other objections to stopcocks are that they are liable to leak unless well ground, and they add considerably to the cost of the apparatus. The increasing necessity of gas analysis in medical diagnosis and in research makes it important that simple and reliable apparatus be available.* Such an apparatus can be constructed by using screw clips in place of stopcocks, provided some means be taken to adjust the gas pressure in the burette after the screw clips have been tightened. This can be accomplished by the use of the "pressure adjuster" described below.

Another difficulty which the inexperienced constantly meet with in using gas burettes of the usual pattern (Haldane's) is in preventing the strong alkaline solution from running up the narrow tubing which connects the burette and absorption bulbs. This accident delays the analysis, since all traces of the alkali must be removed from the burette and mercury before proceeding with the analysis, and moreover the alkali, if not removed, eats its way around the stopcock, and causes etching of the glass and "freezing." In the following apparatus this danger is guarded against by inserting a small bulb on the connecting tubing. The adjustment of the mercury—very little of which is required—is simplified by using a screw clip and pinchcock on the tubing connected with the leveling burette.

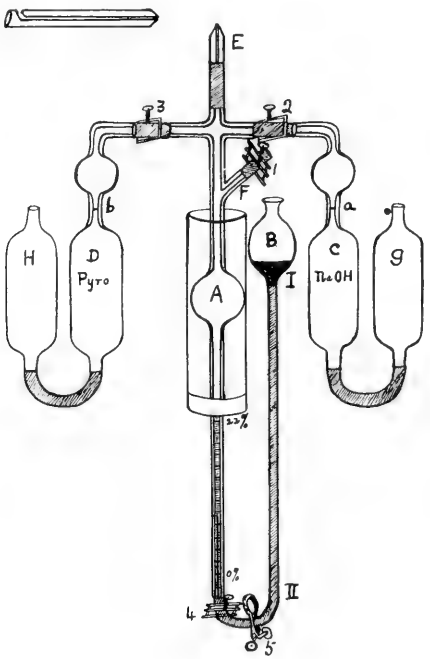
### DESCRIPTION OF THE APPARATUS

The gas burette (*A*) is 10 c.c. capacity from the end of the oblique side tube (*F*) to the lowest graduation on the narrow portion. The uppermost graduation corresponds to 2.2 c.c. from the lowest, the distance between the two being divided into c.c. and 1/50th c.c. The bulb part of the stem to just above the 2.2 mark is surrounded by a water jacket. Above the side tube (*F*) the burette is continued into a narrow-bored tube (narrower than represented in the diagram), with two arms at right angles to each other. These are connected by thick-walled pure-gum tubing, with the absorption bulbs (*C* and *D*) containing re-

*It is not suggested that the apparatus herein described should be employed by those accustomed to the use of the standard burettes or where expense is no object.

spectively a 20 per cent solution of NaOH and a 60 per cent solution of KOH with 10 per cent pyrogallic acid dissolved in it.*

Each bulb is connected below by rubber tubing with the overflow bulbs (*G* and *H*). A small bulb (relatively smaller than represented in the diagram) is blown on the stem of each absorption bulb to serve as a trap preventing the solutions from passing over into the burette. The vertical tube above the cross tubes is closed by the *pressure adjuster,* which consists, as shown in the small side sketch in the diagram, of a glass rod beveled at one end and bored most of the way down the center. At a distance of about 3 mm. from where the beveled portion joins the tube a lateral hole is bored to meet the channel in the center. The adjuster is connected by pure-gum tubing with the central tube of the burette, the beveled end of the former being in contact with the end




of the latter. When the rubber tubing is pinched up opposite the side tube, the burette is brought into communication with the outside and the pressure in it remains undisturbed when the tubing is allowed to fall back into place.

The lower end of the burette is connected by thick-walled rubber tubing with the reservoir (*B*), and on this tubing are a pinchcock (*5*) and screw clip (*4*). About 15 c.c. of mercury suffices to fill the apparatus. The reservoir is hung by a loop of wire around the neck on hooks placed on a wooden upright stand, the higher one being in such a position (marked I†) that the mercury stands at the end of the side tube (*F*), and the lower one (marked II) so that it stands exactly at the mark 0% on the burette.

*NaOH may be substituted for KOH, but is not so satisfactory because of its viscosity. The best solution is made by dissolving pure NaOH (electrolytic, if possible) in an equal weight of water, diluting 10 c.c. of this solution with 4 c.c. water, and dissolving 10 gm. pyrogallic acid in the resulting mixture. Shipley: Jour. Am. Chem. Soc., 1916, xxxviii, 1687.

†The position 1 should be at a higher level than is shown in the figure.

The first step is to fill the tubing between the side tube (F) and the absorption bulbs with nitrogen. This is accomplished by taking a sample of air in the burette by opening screw clip 1, placing the mercury reservoir so that it stands opposite the lower end of the burette (at II), and with the screw clip (*4*) open, cautiously opening the pinchcock (*5*) so that air enters the burette. The screw clip (*2*) is then opened and the reservoir (*B*) raised to position I. By cautiously opening the pinchcock (*5*) the air is made to pass into *C*, and with *5* open the reservoir is raised and lowered several times so that all traces of $CO_2$ are removed from it by the alkaline solution, after which the reservoir is replaced in position II and the pinchcock, previously closed, opened until the NaOH stands at the mark (*a*) on the stem. Screw clip *2* is then closed, *3* opened, and the oxygen removed from the air by repetition of the same procedure. Screw clip *3* is then closed. This preliminary filling of the burette with nitrogen is unnecessary if a previous analysis has been made.

The sample of gas for analysis is now collected in an all-glass (Luer) syringe with the piston well lubricated with vaseline. With the screw clips (1) open, the burette is filled with mercury up to the end of the side tube (*F*), and the nozzle of the syringe is inserted in the rubber tubing of the side tube. While making this connection the piston of the syringe should be gently pressed so that no air may be allowed to become entrapped in the rubber tubing on *F*. To aspirate the sample into the burette the reservoir is placed in position II and the pinchcock *5* opened, gentle pressure being meanwhile maintained on the piston of the syringe. After the gas has been transferred, the mercury meniscus should stand about 1 mm. below the 0 graduation on the burette, this being accomplished by gentle pressure on the piston of the syringe. This leaves the gas in the burette under a slight positive pressure.

The pressure in the burette must now be made equal with that of the atmosphere. For this purpose the pinchcock (*5*) and all screw clips except 1 are opened, and the tubing on the adjuster (*E*) is pinched opposite the bevel. Being under a slight positive pressure, some of the gas in the burette escapes through the adjuster, and it is during this procedure that a slight error is incurred because some of the sample of gas mixes with the nitrogen above the side tube. The error thus incurred is, however, negligible for most purposes. The meniscus of mercury should now stand exactly at the 0% mark.

Screw clip 3 is now closed. This slightly compresses the gas in the burette and lowers the menisci at *a* and *b*, the new level at *a* being marked on the glass by a glass pencil. The reservoir (*B*) is slowly raised and lowered several times (with 4 and 5 open) so that the gas passes in and out of *C*, in which the $CO_2$ is absorbed. In doing this, care must be taken that the mercury does not rise above the level of *F*, and that the NaOH solution does not rise higher than the mark *a*. Four movements usually suffice to absorb all of the $CO_2$.

To determine the volume of the remaining gas, the pinchcock (*5*) is closed, the clip (*4*) half way screwed down, the reservoir placed in position II, and the pinchcock then cautiously opened until the NaOH solution is as nearly

as possible brought back to its original position at *a*. The fine adjustment of this level to the pencil mark is finally effected by means of screw clip *4*. The reading on the burette opposite which the mercury now stands gives in cubic centimeters the amount of $CO_2$ in 10 c.c. of gas. To make certain that all the $CO_2$ has been absorbed, the above procedure should be repeated.

To determine the oxygen, screw clip 2 is closed and 3 opened. The level of the meniscus of the pyrogallic acid solution is marked with a glass pencil at *b*. The gas is then moved in and out of D several times, until all the oxygen is absorbed as determined by no further shrinkage. This takes considerably longer than for $CO_2$. The final volume of gas as read on the burette, less the volume of $CO_2$, gives the oxygen.

After completion of the analysis the apparatus should be left filled with nitrogen and with the screw clips all closed. A short piece of rubber tubing should also be connected with the upper end of the reservoir (*H*) and closed by pinchcocks. This is to prevent undue oxidation of the pyrogallic solution.

As above remarked, a slight error is incurred in using the above apparatus during the adjustment of the pressures. With a little practice, however, this becomes very small. It is very important to make certain that all the rubber unions are perfectly tight, which is best insured by wiring the tubing.

The following analyses for $CO_2$ made by Mr. Shen will serve to illustrate the degree of accuracy of the method.

1. Mixture of nitrogen and $CO_2$ from stock bottle: $CO_2$, 1.78, 1.79, 1.81, 1.76; average, 1.785. Another mixture: 1.91, 1.99, 1.95, 1.95; average, 1.950.

2. Samples of alveolar air taken in all-glass syringe by using the Haldane tube:

a. Deep expiration after a normal inspiration: 6.03, 5.88, 6.04, 6.09, 5.96; average, 6.00.

b. Deep expiration after a deep inspiration: 5.42, 5.38, 5.33, 5.23, 5.54; average, 5.38.

3. Samples of air aspirated from the mouth after a deep expiration. This was done by placing a short piece of rubber tubing attached to an all-glass syringe at the back of the mouth, closing the lips around it after making a quick forced expiration, and quickly withdrawing the piston, the "dead space" of the tubing having been filled with alveolar air by a preliminary trial of the procedure.

a. Deep expiration after a normal inspiration: 5.76, 6.07, 6.09, 6.10; average, 6.00.

b. Deep expiration after a deep inspiration: 5.42, 5.31, 5.44; average, 5.39.

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# ADRENALIN VASODILATOR MECHANISMS

FRANK A. HARTMAN, LESLIE G. KILBORN and LOIS FRASER

*From the Department of Physiology, University of Toronto*

Received for publication May 24, 1918

From the results of recent work regarding the vasomotor reaction to adrenalin several facts seem to have been established. It has been found that in anaesthetized cats and dogs the arterioles supplying skeletal muscle dilate when small quantities of adrenalin are injected into the circulation and that their reaction changes to constriction when the concentration of adrenalin is sufficiently increased (1), (2). The vessels of the intestinal tract have been found to give the opposite response since they constrict when small, and dilate when large doses are injected (3). It has been shown that there are many parts of the organism, bone (4), skin (5), spleen (3), (6), and possibly kidney (3), (7), the vessels of which show no active dilatation from doses of any strength. It is further conceded by the most recent workers (2), (8),(9) that all blood vessels which are dilated by small quantities of adrenalin lose this reaction, for the time being at least, when separated from central control by cutting their nerves. Dilatation under these circumstances is generally replaced by constriction. The reason for this change in reaction and the whole question of the mechanisms involved are still debated. It has been repeatedly suggested that the conflicting effects of adrenalin in varying concentration are entirely due to its stimulation of neuromuscular junctions of two kinds, one constricting and the other dilating. Those who hold this view believe that vessels which have been recently denervated fail to respond by dilatation to adrenalin because of loss of tone (9), (10).

Work from this laboratory has shown that stimulation of the sympathetic and dorsal root ganglia by adrenalin is sufficient to account for the dilatation (11). Although Gruber has shown that some time after denervation peripheral mechanisms respond in a similar manner, this might be due to loss of sensitivity by the constrictor myoneural junctions. The present research is an investigation of this problem.

## METHODS

The methods employed are those of the previous researches described in this Journal, with some modifications and additions. Adrenalin chloride solution (Parke, Davis & Company) was used except in one experiment, in which a more concentrated solution was needed for direct application to ganglia. In this case we used pure adrenalin, made by the same firm. Blood pressure was taken from the carotid artery and injections into the general circulation were made by way of the jugular vein. In order to reduce the constrictor effects of the skin, we eliminated the paw by using a metal cuff open at both ends, a side-tube furnishing connection for the bellows. Both ends of the cuff were made air-tight by packing with a vaseline-cotton or vaseline-paraffin-cotton mixture. In the perfusion experiments, when records were to be taken of one hind limb only we put the cannula into the common iliac artery; when both limb volumes were being recorded we perfused through the abdominal aorta immediately above the bifurcation. The perfusion fluid was allowed to escape through slits in the iliac vein or veins directly into the abdominal cavity since any attempt to lead it away through cannulae from the veins resulted sooner or later in clotting. In some experiments we had difficulty in getting an equal flow of the perfusion solution to the two limbs. Results from these were of course discarded. The difficulty was found to be lessened by tying the internal iliac and the middle sacral arteries. The pressure employed for perfusion varied in different experiments between 10 mm. and 50 mm. Hg., the average being about 20 mm.

In all experiments involving denervation of a limb both the sciatic and femoral nerves were severed. Aseptic precautions were observed in those animals which were to be kept for later use. In none of our experiments did infection of the muscles result. The skin suppurated in a few cases due to post-operative infection, but this seemed in no way to affect the muscle.

In the two experiments (p. 505) in which the changing volume of a limb after denervation was to be continuously recorded as well as its response to periodic doses of adrenalin, we connected the plethysmograph by means of a T-piece to two bellows, one large and one small. The slow changes were recorded on the larger one, while the little one (deflated) was clamped off. When the time came for injection, the clamp was removed from the small bellows tube, the latter bellows being slightly inflated by a small compression of the larger bellows, then the

tube leading to the large bellows was clamped. The small bellows was thus prepared to register a small volume change in the limb. After the injection effects were finished, the clamp on the large bellows tube was removed, the small bellows was deflated by forcing the air into the large bellows and then clamped off. By this method no air was lost during the experiment.

## RESULTS

*Response after recent denervation.* The peripheral effect of adrenalin was compared with the total "gangliar peripheral" effect in fifteen cats

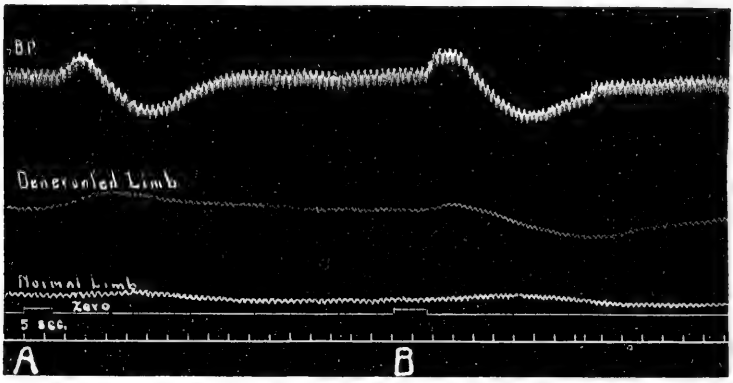


Fig. 1. Small active dilatation (A) of a denervated limb which occurs when 0.2 cc. of adrenalin, 1: 100,000 is given disappears when a slightly larger dose 0.4 cc. of the same solution, (*B*) is injected. Although the bellows for the normal limb was less sensitive, it does show that the maximum dilatation of the normal limb coincides with the maximum fall in blood pressure while the dilatation of the denervated limb does not coincide. Cat, 3.3 kgm. (Reduced one-half)

by studying the volume changes in a denervated limb simultaneously with those in a normal limb (2). The response of the denervated limb was predominantly constriction, although there was a short period of dilatation which usually occurred at the time of the blood pressure rise. Except in a few instances this was undoubtedly a passive effect. In these the dilatation persisted for a short time during the blood pressure fall and came earlier than that in the normal limb (fig. 1, *A*). This dilatation occurred only from small doses of adrenalin, a small increase in the adrenalin being sufficient to obliterate all but a slight passive effect (fig. 1, *B*). On the other hand more than ten times the dose of

adrenalin was required to produce constriction in the normal limb as compared with that for constriction in the denervated limb, e.g., constriction in the denervated limb always occurred with doses of about 0.2 cc. to 0.4 cc., 1: 100,000 adrenalin or less, while from 0.3 cc. to 1.0 cc. 1: 10,000 adrenalin was necessary to produce a similar result in the normal limb.

Cutting the nerves to the limb must produce the result described either by removing the influence of the gangliar dilator mechanism or by modifying the blood vessels themselves so that they do not respond through the medium of the peripheral mechanism. From Gruber's work it appears that after some time has elapsed the dilator response to adrenalin develops in the denervated limb. He assumes that this is due to a recovery of tone. In order to test this theory we conducted the following experiments.

After both the sciatic and femoral nerves of one hind limb were dissected out and secured by loose ligatures, the limb was placed in a plethysmograph tube connected to the double bellows system described above. The nerves were severed and the change in volume of the leg registered every five minutes during the remainder of the animal's life. Every hour the response to a depressor dose of adrenalin was determined. In this way the adrenalin reaction could be studied in direct relation to the condition of relaxation or contraction of the vessel walls.

In the first experiment of this kind the animal (cat, 1.8 kgm.) was anaesthetized with ether and lived for eight hours. The limb dilated at an almost uniform rate for the first five hours after the nerves were cut. Dilatation became slower during the sixth hour and had completely stopped at the end, from which time the volume of the limb remained the same until the eighth hour, when the animal died. The blood pressure remained fairly good until a short time before death. The dose of adrenalin used for testing was 0.2 cc., 1: 100,000. Throughout the experiment this produced a fall in blood pressure, preceded by a slight rise. The limb responded by a short dilatation (which may easily have been due to the preliminary blood pressure rise) followed by a more prolonged constriction until the end of the sixth hour when active dilatation appeared. In other words while the limb was in the process of dilating as a result of denervation adrenalin caused constriction, but when the dilatation from this cause was complete a small amount of active dilatation occurred from adrenalin.

In a second experiment where urethane was given, the cat (2.2 kgm.) lived thirty-three hours. The maximum dilatation was reached be-

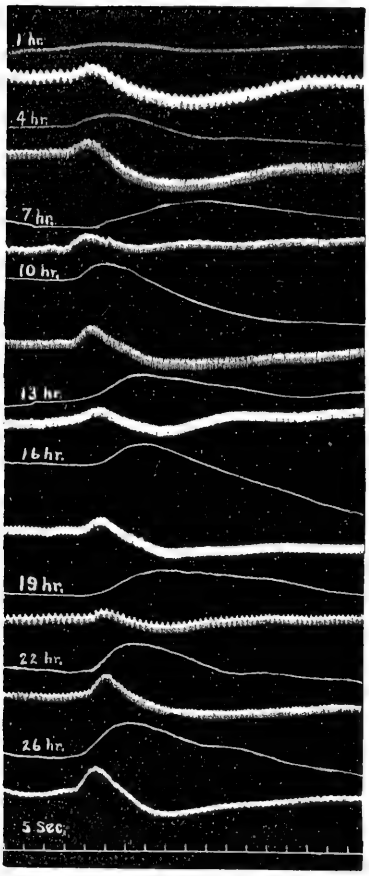


Fig. 2. The response of a denervated limb to a depressor dose of adrenalin during "atonic" and "tonic" conditions. The hours represent the length of time after cutting the nerves. The period of maximum dilatation was reached between the sixth and seventh hours. Up to that time the vessels may be considered "atonic;" after the seventh hour they may be considered "tonic;" 0.5 cc., adrenalin 1: 100,000 was injected in each case. The upper record at each hour represents limb volume, the lower is blood pressure. Cat, 2.2 kgm. Urethane. (Reduced one-half)

tween the sixth and seventh hours. It did not remain long at this level, but constriction soon began, continuing gradually until the twenty-second hour, when it ceased. It remained at this level for the next eight hours. The amount of this remaining dilatation was about one-fifth of the maximum. The dose of adrenalin in each instance was 0.5 cc., 1: 100,000. This usually produced a fall in blood pressure, which was preceded by a rise. During the first five hours adrenalin produced dilatation and constriction of the limb, the dilatation appearing to be largely passive. At the sixth and seventh hours the dilatation became more active and from that time onward the dilator reaction to adrenalin was more pronounced. This was undoubtedly due in part at least to active stimulation, although there was considerable variability in the curves, sometimes the constriction being more pronounced and the dilatation more passive (fig. 2). On the whole it may be said from the two experiments that active dilatation of a denervated limb in response to adrenalin becomes more prominent after the relaxation resulting from denervation has ceased.

In the above experiment we found that a large part of the dilatation resulting from denervation had been recovered from in eighteen hours and that there was little change for the next twelve hours. At this time if the nature of the reaction depends on the condition of tone in the vessels, adrenalin should give good dilatations. In addition to the experiment just described we tried two others. One

hind limb was denervated in each of two cats. Eighteen hours later the animal was again anaesthetized with ether and a study made of the adrenalin response, with the following results:

*Cat, 2.2 kgm.*, 0.3 cc., 1: 100,000 adrenalin caused a similar amount of dilatation in both the normal and denervated limbs. Doses of 0.5 cc. to 1.0 cc., 1: 100,000 adrenalin produced either constriction alone or else dilatation and constriction in the denervated limb. Larger doses produced marked constriction in the same limb. Doses as large as 5.0 cc., 1: 100,000 still produced dilatation in the normal limb, moreover these dilatations were much more pronounced than any resulting in the denervated limb. It took 0.8 cc., 1: 10,000 adrenalin to cause a reversal in the normal imb and then it was not complete, dilatation preceding the constriction.

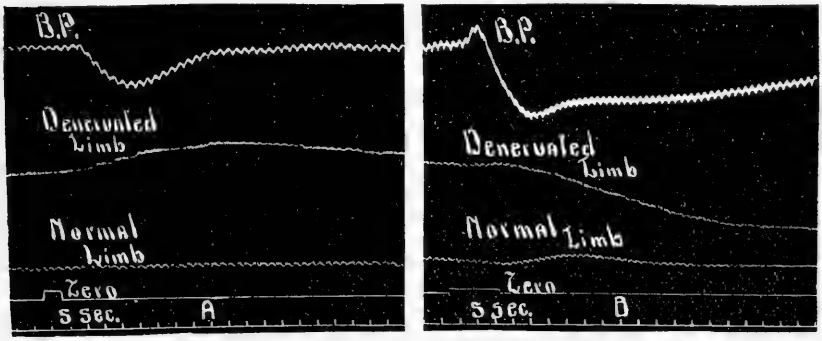


Fig. 3. *A*. Marked active dilatation of a denervated (18 hr.) limb with a small dose of adrenalin 0.2 cc., 1: 100,000. No effect in the normal limb. *B*. Constriction of the same denervated limb with 1.5 cc., 1: 100,000 adrenalin; dilatation of the normal limb. Cat, 2.6 kgm. (Reduced one-half)

*Cat, 2.6 kgm.*, 0.2 cc., 1: 100,000 adrenalin caused a marked dilatation in the denervated limb, but no effect in the normal limb (fig. 3, *A*). Dilatation in the denervated limb, occurred with doses as large as 1.0 cc., 1: 100,000 but 1.5 cc. of the same concentration caused constriction (fig. 3, *B*). Dilatations were not produced in the normal limb until 0.3 cc., 1: 100,000 adrenalin was injected. Dilatation in this limb resulted from doses as large as 0.5 cc., 1: 10,000 adrenalin; however, 0.7 cc. of the latter concentration caused a reversal.

In both experiments the range of dosage for dilatation in the denervated limb was small while quite large amounts of adrenalin were required to bring about reversal in the normal limb. It seems from these experiments that tone may play a part in the response of a denervated limb to small doses of adrenalin. Moreover it appears that the

TABLE 1

*A comparison of normal and denervated limbs*

| ANIMAL | WEIGHT | DURATION OF DENERVATION | DOSE | RESPONSE OF NORMAL LIMB | RESPONSE OF DENERVATED LIMB |
|---|---|---|---|---|---|
| | *kgm.* | *days* | *cc.* | | |
| 1. Cat | 2.4 | 7 | 0.6 A | Dilatation* | Dilatation |
| | | | *1.0 A* | *Dilatation and constriction* | Dilatation |
| | | | *0.2 B* | *Marked constriction* | Dilatation |
| | | | *0.5 B* | *Very marked constriction* | Dilatation |
| | | | *1.0 B* | *Very marked constriction* | *Dilatation and constriction* |
| 2. Cat | 3.0 | 14 | 0.3 A | Dilatation | Dilatation |
| | | | *0.4 A* | *Constriction* | Dilatation |
| | | | *0.7 A* | *Constriction* | *Constriction* |
| 3. Cat | 2.2 | 15 | 0.4 A | Slight dilatation | Dilatation |
| | | | 0.5 B | Slight dilatation | *Marked constriction* |
| | | | *1.0 B* | *Slight constriction* | *Marked constriction* |
| 4. Dog | 14.0 | 22 | 1.0 A | Dilatation | Dilatation |
| | | | 1.6 A | Marked dilatation | Marked dilatation |
| | | | *2.5 B* | *Dilatation and constriction* | *Dilatation and constriction* |
| 5. Dog | 6.2 | 31 | *0.2 A* | *Nothing* | Dilatation |
| | | | *0.5 A* | *Dilatation and constriction* | Dilatation |
| | | | *0.2 B* | *Dilatation and constriction* | Marked dilatation |
| | | | *0.5 B* | *Very marked constriction* | *Marked dilatation and marked constriction* |
| 6. Dog | 5.6 | 39 | 0.2 A | Slight dilatation | Slight dilatation |
| | | | 1.5 A | Dilatation | Dilatation |
| | | | 5.0 A | *Dilatation and constriction* | Very marked dilatation |
| | | | 1.0 B | *Dilatation and constriction* | *Dilatation and constriction* |

* Unless otherwise stated dilatation means active dilatation.

A = 1: 100,000 adrenalin.

B = 1: 10,000 adrenalin.

peripheral mechanism has a much more limited action than the "gangliar-peripheral" mechanisms when taken together.

*After denervation of greater duration.* Animals (six dogs and three cats) were studied which had had the sciatic and femoral nerves severed

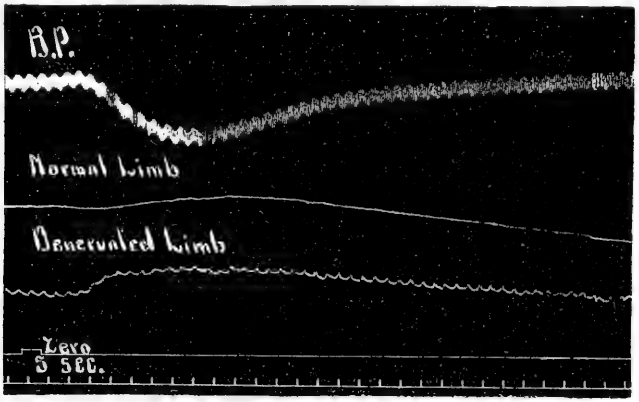


Fig. 4. Dilatation of the hind limb of a cat (2.4 kgm.) to 0.2 cc., adrenalin, 1: 100,000, seven days after denervation. (Reduced one-half)

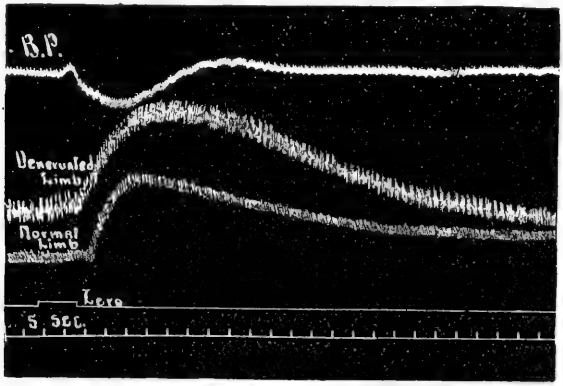


Fig. 5. Dilatation of the hind limb of a dog (14 kgm.) to 0.8 cc. adrenalin, 1: 50,000, twenty-two days after denervation. (Reduced one-half)

in one limb from seven to thirty days before. It can be seen from the following table (table 1) that although the lapse of a week in most cases renders the peripheral dilator mechanism more effective (see figs. 4 and 5), a greater amount of time does not materially increase the

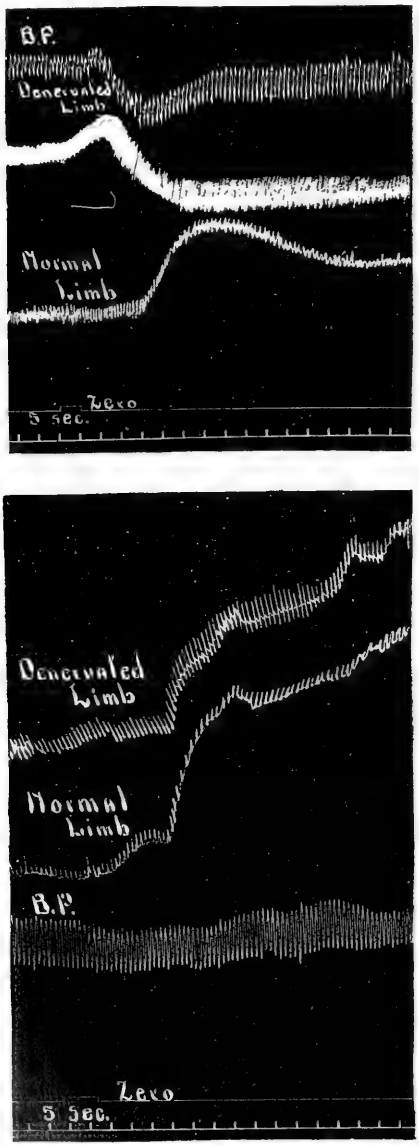


Fig. 6. Reversal of the adrenalin response in a freshly denervated limb by perfusion. Upper record—circulation to limbs intact, 1.0 cc. adrenalin, 1: 10,000 injected into the jugular vein. Lower record—limbs perfused, 2.0 cc. adrenalin, 1: 10,000 injected into the perfusion fluid. Dog 24 kgm. (Reduced one-half)

TABLE 2

*Comparison of normal and denervated limbs before and after perfusion*

| ANIMAL | WEIGHT | DOSE OF ADRENALIN | NORMAL LIMB | DENERVATED LIMB |
|---|---|---|---|---|
| | *kgm.* | *cc.* | | |
| 7. Dog | 17.0 | 0.5 A | Dilatation | |
| | | 0.3 B | Dilatation | |
| | | 0.7 B | Constriction | |
| | | *1.0 A* | *Dilatation* | |
| | | *1.0 B* | *Dilatation and constriction* | |
| 8. Dog | 15.0 | 1.3 A | Dilatation and constriction | Small dilatation and marked constriction |
| | | 0.5 B | Marked dilatation and small constriction | Marked constriction |
| | | *0.7 A* | | *Marked dilatation* |
| | | *0.4 B* | | *Dilatation and constriction* |
| | | *3.0 B* | | *Pure constriction* |
| 9. Dog | | 0.5 A | Dilatation | Constriction |
| | | 0.4 B | Marked dilatation | Marked constriction |
| | | 0.7 B | Dilatation and constriction | Marked constriction |
| | | *1.0 A* | | *Dilatation* |
| | | *4.0 B* | | *Marked dilatation* |
| | | *1.0 C* | | *Dilatation and constriction* |
| 10. Dog | 7.5 | 0.4 A | Dilatation and constriction | Dilatation and constriction |
| | | 4.0 A | Dilatation and constriction | Marked constriction |
| | | *0.5 A* | *Dilatation* | *Dilatation* |
| | | *1.0 B* | *Constriction* | *Constriction* |
| 11. Dog | 24.0 | 0.5 B | Dilatation | Constriction |
| | | 2.5 B | Marked dilatation | Marked constriction |
| | | 4.5 B | Dilatation and constriction | Marked constriction |
| | | *1.0 B* | *Marked dilatation* | *Dilatation* |
| | | *5.0 B* | *Dilatation and constriction* | *Dilatation and constriction* |

A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.
C = 1: 1,000 adrenalin.

Limb perfused where italics are used, injections in that case into the perfusion fluid, otherwise into the jugular vein.

effect. In most cases the constrictor mechanism had become less sensitive as compared with that in the normal limb (see animals 1, 2 and 5, table 1). On the other hand, occasionally the dilator mechanism was easily fatigued so that after a few doses the dilator response disappeared or was considerably decreased.

TABLE 3

*Comparison of perfused limbs of animals in table 1**

| ANIMAL | WEIGHT | DOSE | NORMAL LIMB | DENERVATED LIMB |
|---|---|---|---|---|
| | *kgm.* | *cc.* | | |
| 4 Dog denervated 22 days | 14.0 | 2.0 A | Dilatation | Dilatation |
| | | 0.4 B | Dilatation | Dilatation |
| | | 1.5 B | Dilatation and constriction | Dilatation and constriction |
| 5. Dog denervated 31 days | 6.2 | 0.05 A | No effect | No effect |
| | | 0.1 A | Small constriction | Small constriction |
| | | 0.5 A | Dilatation and constriction | Dilatation and constriction |
| | | 0.2 B | Dilatation and marked constriction | Dilatation and small constriction |
| | | 0.2 B | | Marked dilatation |
| | | 0.5 B | | Very marked dilatation |
| | | 1.0 B | | Very marked dilatation |
| | | 0.5 C | | Marked dilatation |
| | | 0.8 C | | Dilatation and constriction |

* Injections into the perfusion fluid.
A = 1: 100,000 adrenalin.
B = 1: 10,000 adrenalin.
C = 1: 1,000 adrenalin.

The dilatation of the denervated limb was no better developed in these animals than in some of the responses from a limb denervated but a few hours before (see fig. 2; 7 hr., 19 hr.). However the dilatation was more constant in occurrence and resulted from a greater range of doses. From the very fact that dilatation quite often takes place in the denervated limb from doses larger than those necessary to produce reversal in the normal limb, it seems that a change has taken place in

the myoneural junctions. The constrictor junctions must have lost in sensitiveness or the dilator junctions have gained.

*Response of perfused limbs.* We have obtained dilatation of both normal and denervated limbs from the injection of adrenalin into the fluid which was perfusing them. A comparison of the perfused normal and denervated limbs injected in this way should help to explain the peripheral dilator mechanism.

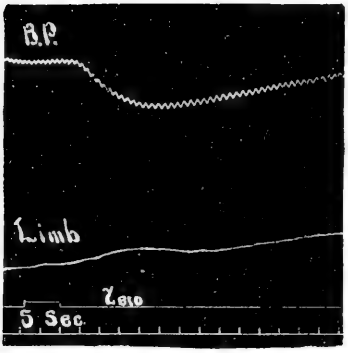


Fig. 7

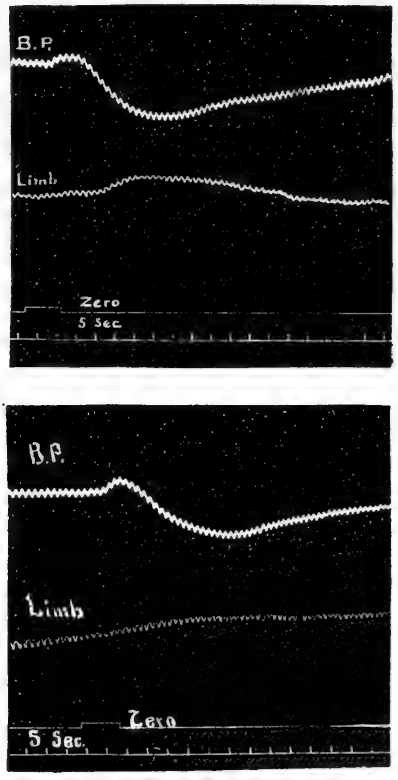


Fig. 8

Fig. 7. Dilatation of a perfused hind limb of a cat by the action of a depressor dose of adrenalin (0.6 cc., 1: 100,000) upon the gangliar portion of the dilator mechanism. Cat 2.4 kgm. (Reduced one-half)

Fig. 8. Dilatation of a hind limb produced by a depressor dose of adrenalin acting upon the gangliar portion of the dilator mechanism. Upper record—response of the hind limb to 0.4 cc. adrenalin, 1: 100,000 injected into the jugular vein, circulation intact. Lower record—response of the same limb to 0.6 cc. adrenalin, 1: 100,000 injected into the jugular vein. (Reduced one-half)

By perfusion of a recently denervated limb an immediate change in the response to adrenalin is brought about, so that dilatation instead of constriction is easily produced (fig. 6 and table 2). This change is similar to that occurring in a denervated limb with normal circulation several hours after denervation (figs. 2, 3, 4, 5 and table 1). The peripheral response to adrenalin in perfused normal and denervated limbs is essentially the same when carried out simultaneously in one animal (animals 10 and 11, table 2). On the other hand there is greater variability in the response of a perfused limb which has been denervated for several days. In one case the constrictors were easily fatigued so that after a few doses of adrenalin they could not again be brought into action except by a dose of 0.8 cc., 1: 1,000 (animal 5, table 3). In another case even perfusion did not bring about dilatation in an animal which had shown no active dilatation with intact circulation (dog, limb denervated eight days). The normal limb gave dilatation before and after perfusion but this is an exceptional case in our experience.

*"Gangliar" dilatation from depressor doses.* Gruber (9, p. 311) failed to obtain dilatation of a perfused limb from the injection of depressor doses of adrenalin to the general circulation. He infers that the gangliar effect is produced only by pressor doses. We have been able to show in two experiments that depressor doses of adrenalin can bring the gangliar mechanism into action. In both animals a slight increase in the dose was necessary, but the blood pressure response was a pure fall or else a slight rise and decided fall. The animals were cats weighing 2.4 kgm. and 3.0 kgm. In the first, 0.6 cc., 1: 100,000 was required after perfusion (fig. 7). In the second, 0.4 cc., 1: 100,000 caused dilatation before, while 0.6 cc., 1: 100,000 was required after perfusion (fig. 8). When perfusion had gone on for some time even larger doses of adrenalin were required to produce dilatation.

## DISCUSSION

*The relation of tone to the reversal of adrenalin effects.* Recognizing the fact that adrenalin may cause dilatation through both gangliar and peripheral action, we are confronted with the question as to the normal site of dilator action. It has been shown that cutting gangliar connection with the limb in a majority of cases prevents the dilatation of that part. Gruber (9, p. 307) maintains that this is due to a loss of tone in the vessels. In order to understand the development of the tone theory, we should first consider the work of Cannon and Lyman

(10) who were the first to suggest this interpretation for the opposite effects of depressor doses of adrenalin. Their view was reached by the exclusion of other possibilities, viz., (1) central source, (2) blocking of vasoconstrictor impulses, (3) stimulation of vasoconstrictor and vasodilator nerve endings. Their exclusion of the third possibility was on account of the meagre evidence for the existence of vasodilator nerves in the sympathetic system. They found that the blood pressure response was changed to a rise if the tone had been lowered sufficiently by overheating, separation from the central nervous system or by extreme action of the depressor nerve. They attributed vasodilation and vasoconstriction to opposite actions of adrenalin according to the state of the muscle—relaxation when tonically shortened, contraction when relaxed.

Gruber's conclusions were reached because of his inability to obtain dilatation in a freshly denervated limb and the recovery of the dilator response in a limb a few days after denervation. He attributed the reappearance of the dilator reaction to a restoration of tone. It might also be due to a loss in sensitiveness of the constrictor myoneural junctions.

Let us consider, first, the question of tone. In all of our experiments with recently denervated animals the reactions to adrenalin were studied within thirty minutes after denervation and were continued for one or two hours. We have shown above that the maximum dilatation is not reached until the sixth hour after denervation so that those studies were made during the period of steady relaxation. Within this time the usual adrenalin response is constriction, afterwards the reaction begins to reverse (fig. 2). It is not that the vessels have suddenly dilated to their limit and cannot expand further, because they only gradually reach this stage after six or seven hours. Moreover they do not appear to dilate to the limit at any time as a result of denervation because while they are in this state of maximum relaxation, depressor doses of adrenalin often cause further dilatation (fig. 2). We may draw the conclusion that while relaxation is going on the vasodilator myoneural junction is not so easily brought into action and that the constrictor effect therefore predominates.

The state of relaxation seems to affect only the adrenalin receptive substance. Active dilatation of a denervated limb in which the vessels are relaxing can easily be produced by a substance from ox pituitaries (fig 9). In the same animal depressor doses of adrenalin usually caused constriction of the denervated limb (fig. 1).

*After denervation of greater duration.* A few days after cutting the nerves to a limb, the latter has regained its power to dilate in response to adrenalin so that it does as well as the normal limb. This might be explained by the recovery in tone, but a large part of the tone has been recovered within twenty-four hours, so that the reaction at the twenty-fourth hour should not differ much from that several days later. But it does differ in this respect that in denervations of longer duration it requires much larger doses of adrenalin to cause constriction; in other words, there is a larger range of dosage producing dilatation. In fact a larger dose than that required for the normal limb is needed to bring about reversal in the majority of cases (table 1). Gruber (9, p. 310) also found this to be true. One can interpret this either as a loss in sen-

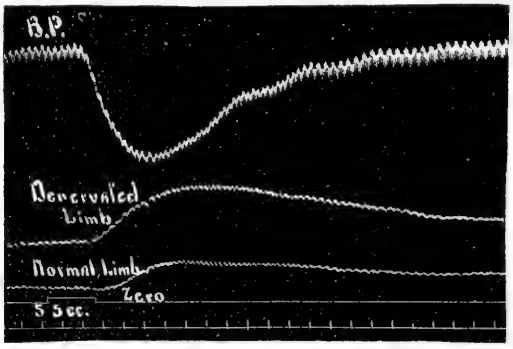


Fig. 9. Dilatation of a freshly denervated limb, produced by a depressor substance obtained from pituitary glands. Cat. (Reduced one-half)

sitiveness of the constrictor junctions or a gain in sensitiveness of the dilator junctions. The tone theory, however, does not appear to account for this point.

In regard to the question of variation in sensitiveness of the myoneural junctions we have the work of Elliott (12), which indicated that all muscles thrown into contraction by adrenalin have their irritability to this substance increased by denervation. However we have no proof that dilator junctions would be thus affected.

*Effects of adrenalin in perfused limbs.* A number of investigators have studied the response of various perfused organs to adrenalin with variable results. This would be one of the best methods of proving the existence of vasodilator nerves in the sympathetic if active dilatation could be so obtained.

Employing the change in rate of venous outflow to indicate the vasomotor response Salvioli (14) and Brodie and Dixon (15) obtained only constriction in the hind limb when adrenal extract or adrenalin was added to the perfusion fluid. The latter experimenters found this to be true even in limbs which had been denervated two or three months before. Pari (16) repeatedly obtained an increased outflow from the limb in one experiment when a perfusion of 1:500,000 adrenalin was used, but he inferred that this was due to decomposition products.

Langendorff (17) from his results with rings of coronary arteries concluded that they possessed sympathetic vasodilators which were stimulated by adrenalin. His results were confirmed by Cow (18) and Park (19). Brodie and Cullis (20) from experiments upon perfused hearts concluded that the main cause of adrenalin dilatation was the excitation of vasodilator "nerve-endings."

Langlois and Desbouis (21) obtained constriction in the lung vessels with large doses, 1.0 mgm., and dilatation with small doses, 0.05 mgm. Similar results on perfused lungs were described by Tribe (22).

Other organs have given dilatation from dilute adrenalin perfusing them. For instance, the kidney and the intestine have been found by Ogawa (23) to react in this way. But so far as we know the limb has not been found to react thus when perfused except in the one experiment of Pari (16) and in experiments by Ogawa (23) on the rabbit in which he sometimes obtained dilatation following constriction, but never primary dilatation.

We found it easy to produce dilatation by the injection of adrenalin into the fluid perfusing a limb. Whether the nerves had been cut or not seemed to make no difference in the reaction (table 2).

Why should perfusion reverse the reaction of a denervated limb? Does it mean that perfusion of vessels which were previously relaxing causes them to begin to contract and thus produces the reversal? That might be the condition in perfusion with low pressure (20 mm.) but in a number of our experiments we have doubled or tripled the pressure without materially reducing the dilator response to adrenalin. Moreover Tribe (22) found in the perfused lung that with high pressure it was easier to obtain dilatation than constriction.

Another observation which suggests an explanation of the results just described is the increase in the range of doses of adrenalin which will cause dilatation in both normal and denervated limbs. The interpretation which this seems to suggest is that perfusion renders the constrictor myoneural junction less sensitive or the dilator junctions more

sensitive. We have found that it takes much larger doses of adrenalin to bring about constriction in a perfused limb than it did while the circulation was intact, whether it be a denervated limb or one with nervous connections (table 2). For example: whereas 1.0 cc., 1: 10,000 adrenalin injected into the jugular vein before perfusion caused constriction in the denervated and dilatation in the normal limb, 2.0 cc., 1: 10,000 (a dose more than four times as great, considering the limited circulation of the perfusion fluid) injected into the perfusion fluid caused marked dilatation in both limbs (fig. 5). Mechanical effects from the injection were compensated for by a simultaneous withdrawal of an equal quantity of perfusion fluid.

The work of Meyer (13) supports the idea that Ringer's solution modifies the sensitiveness of blood vessels to adrenalin. He found that artery rings kept for some time lost their sensitiveness to adrenalin from day to day and after it had disappeared an opening shock still produced contraction. His results might be due to the changed medium in which the preparations were kept rather than to denervation.

*Dilatation from the stimulation of "gangliar" and "peripheral" mechanisms.* Before we enter into the discussion of the relative importance of the "gangliar" and "peripheral" mechanisms we wish to call attention to the results of Gruber (9, p. 311), in which he failed to obtain dilatation of a perfused limb from the injection of a small dose of adrenalin into the general circulation. Because the same dose caused dilatation in the intact limb he infers that the dilatation from small doses must be due to peripheral instead of gangliar action. He says:

> If adrenalin exerted its influence entirely through a vasodilator center, it should produce the same results in these two cases where the only difference in the conditions of the limbs is that one has and one has not the circulation intact.

This is a very serious difference and might easily account for the increase in the dilator threshold. Oxygenated Ringer's solution or even oxygenated defibrinated blood cannot be expected to fulfil the function of normal blood in all respects and indeed this was not the only difference, for the occlusion of the abdominal aorta interferes with the circulation to the ganglia of the nerves supplying the limbs. The latter condition alone might necessitate a larger dose of adrenalin. If both of these conditions were operative, the dose required would probably in many cases be a pressor dose. However, we have been able to show in two experiments that depressor doses can bring the gangliar mechanism into action. These render unnecessary the assumption of periph-

eral action to account for dilatation resulting from small doses of adrenalin.

We are not in a position to say which is more important in producing dilatation normally, the "gangliar" mechanism or the myoneural junction. It has been possible in some animals to obtain the same amount of dilatation by the action of adrenalin upon the gangliar portion of the mechanism alone (limb perfused, adrenalin injected into the jugular vein) as occurred from the injection of the same quantity when the circulation of the limb was intact. In many cases, however, larger doses are required to produce equal response in the limb when only the gangliar mechanisms are affected as compared with the condition where both gangliar and peripheral portions might be brought into action. This may easily be attributed to the reduced circulation to the ganglia brought about by clamping the aorta high in the abdomen, but the fact that the peripheral dilator mechanism can be brought into action rather easily under many circumstances indicates that it may well be as important as the gangliar dilator mechanism. At any rate we seem justified in concluding that sympathetic vasodilators to the limb exist and that they are sensitive to adrenalin at the "gangliar" and "peripheral" ends.

We wish to thank R. S. Lang for assistance in this research.

## SUMMARY

1. While a limb is dilating from denervation adrenalin produces an increase in volume with difficulty, but while the reverse change is taking place the dilator effect of adrenalin begins to reappear.

2. After denervation of a limb, of greater duration, the dilatation from adrenalin occurs from a greater range of doses than is the case in the normal limb.

3. The peripheral action (dilatation) becomes similar in both normal and denervated limbs after perfusion. Under these conditions also dilatation occurs with a greater range of doses.

4. Depressor doses of adrenalin can cause dilatation of a limb by action on the gangliar mechanism.

5. Adrenalin acts on both "gangliar" and "peripheral" mechanisms in producing dilatation of the hind limb.

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# CONSTRICTION FROM ADRENALIN ACTING UPON SYMPATHETIC AND DORSAL ROOT GANGLIA

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In the preceding research it has been shown that adrenalin can produce dilatation in a limb by acting upon a "peripheral" mechanism as well as upon a gangliar mechanism. We have been able to show that the constrictor action of this hormone is not confined to the myoneural junction. Although the gangliar response is not easily obtained, it has been found often enough to draw our attention. The methods employed were those described in preceding researches.

All experiments showing constriction from gangliar action must necessarily be those in which the organ tested is completely cut off from the general circulation in order to prevent the peripheral action of adrenalin.[1] Perfusion experiments in which anastomoses to the organ are cut off, satisfy this condition.

*Constriction of the limb.* Six animals out of nineteen furnished evidence of gangliar constriction in the hind limb. One dog (16 kgm.) and one cat (3 kgm.) gave constriction followed by dilatation when adrenalin was injected into the jugular vein; the first with a dose of 4 cc., 1: 20,000 adrenalin, the second with a dose of 5 cc., 1: 5,000 adrenalin. In each animal both sympathetic and dorsal root ganglia were intact. On the other hand similar experiments with six dogs and three cats gave no constriction although the usual dilatation could be obtained.

*From sympathetic ganglia.* Two cats gave positive evidence of a constrictor action of these ganglia by the direct application of adren-

[1] Salvioli (Arch. ital. de biol., 1902, xxxvii, 384) perfused the limb of a dog, with the nerves intact. Adrenal extract was injected into the jugular vein and the volume change in the limb was studied by the venous outflow. He usually obtained no change in the flow but occasionally there was a small decrease in the outflow. This was believed to be due to the escape of adrenal extract into the limb because the decrease was not synchronous with the rise in blood pressure; in fact the pressure had returned to normal before the limb changed.

alin to them. In the first, a 1: 100,000 adrenalin solution produced only dilatation while a 1: 10,000 solution caused steady marked constriction. In the other a 1: 1,000 solution caused a dilatation followed by constriction. Three animals gave no constriction. The first (a cat) was tried by dropping adrenalin upon the sympathetic ganglia. On the last two (dogs) the dorsal root ganglia had been removed, adrenalin being given by the jugular vein.

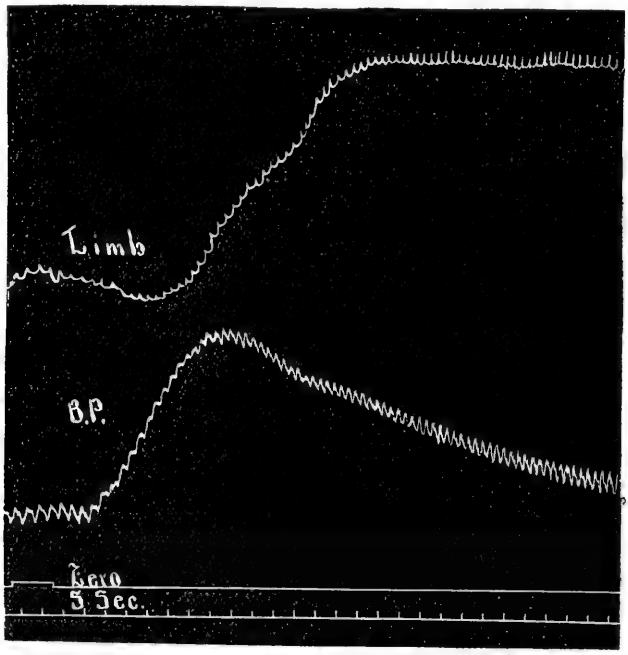


Fig. 1. Constriction and dilatation of a perfused limb from the injection of 4 cc. adrenalin, 1: 5,000 into the jugular vein. All sympathetic ganglia supplying the limb had been destroyed. Dog 21.6 kgm. Reduced ½.

*From dorsal root ganglia.* Of the animals (seven dogs) in which the sympathetic ganglia to the perfused hind limb had been destroyed, only one responded by constriction when adrenalin was injected into the general circulation (fig. 1). Direct application of adrenalin to the dorsal root ganglia in one of two cats caused constriction in the hind limb (fig. 2). In almost all of the animals studied whether giving gangliar constriction or not, dilatation from adrenalin was obtained.

We may say, in general for the hind limb, that the effect of adrenalin on the ganglia is preëminently dilator and that the constriction from this source is insignificant.

*Constriction of the intestine.* Constriction of a gangliar source was more common in the intestine than in the limb. A response of this

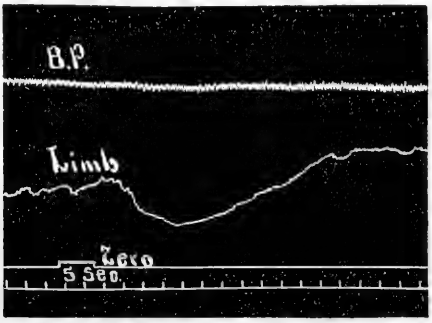


Fig. 2. Constriction of the hind limb resulting from the direct application of 1: 1,000 adrenalin to the lower lumbar dorsal root ganglia. Dog 16 kgm. Reduced ½.

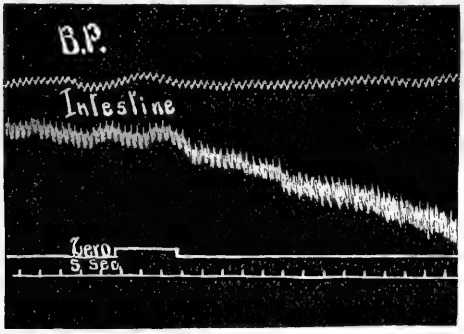


Fig. 3. Constriction of the intestine produced by direct application of 1: 1,000 adrenalin to the twelfth and thirteenth dorsal root ganglia. Dog 11 kgm. Reduced ½.

sort was obtained in six out of thirteen animals. Moreover the number of constrictions obtained in the same animal was much greater in the case of the intestine than in the experiments with the limb. In the latter there would often be only one or two constrictions throughout the whole experiment.

Three dogs whose splanchnic nerves had been cut gave positive evidence of gangliar constriction. The intestinal loop was perfused and the adrenalin was injected into the jugular vein. Both constriction and dilatation occurred whenever the intestine responded by constriction.

Intestinal constriction was also produced by the direct application of adrenalin to the dorsal root and superior mesenteric ganglia.

In a cat although dilatation only had been produced by the application of 1: 1000 adrenalin to the twelfth and thirteenth thoracic dorsal root ganglia in three instances, in a fourth the same concentration produced constriction followed by dilatation.

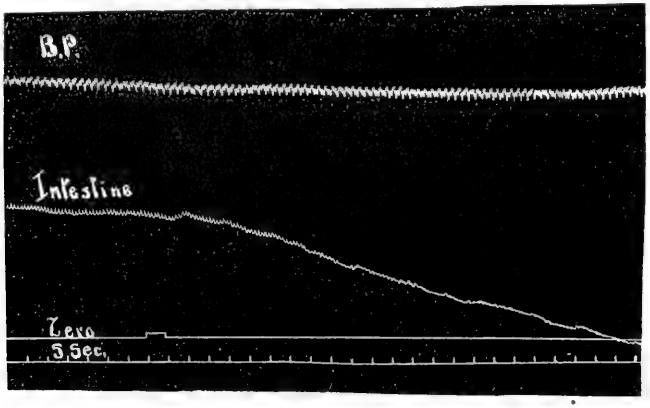


Fig. 4. Constriction of the intestine from direct application of 1: 1,000 adrenalin to the superior mesenteric ganglion. Dog. Reduced ½.

In a dog, a 1: 1,000 solution produced dilatation alone, constriction alone (fig. 3) or constriction followed by dilatation.

Marked constriction of the intestine was caused in another experiment by treating the superior mesenteric ganglion with 1: 1,000 adrenalin chloride to which a little pure adrenalin had been added (fig. 4).

Additional evidence that the superior mesenteric ganglion is a source of constriction was obtained in one animal by the use of nicotine. Before nicotine, adrenalin caused constriction followed by dilatation of the intestine. Intravenous injection of nicotine ruled out both the constriction and dilatation.

Thus the gangliar effect of adrenalin as far as the intestine is concerned is largely dilator, although it is sometimes a source of constriction.

SUMMARY

1. Adrenalin occasionally produces constriction in the hind limb by its action upon the sympathetic and dorsal root ganglia.

2. Constriction of the intestine is sometimes produced by adrenalin acting upon the superior mesenteric and dorsal root ganglia.

# UNIVERSITY OF TORONTO STUDIES

## PHYSIOLOGICAL SERIES

No. 22: THE SPONTANEOUS DEVELOPMENT OF AN ACIDOSIS CONDITION IN DECEREBRATE CATS, BY PROFESSOR J. J. R. MACLEOD

(REPRINTED FROM PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, VOL. XVI)


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19 (1394)

# The spontaneous development of an acidosis condition in decerebrate cats.

By J. J. R. Macleod.

[*From the Physiological Department, Toronto University, Canada.*]

Investigations of the nature of the control of the respiratory center are rendered difficult because of the extreme susceptibility of the center to anesthetics. Much of the recent work has accordingly been done on man by methods suggested by Haldane and his pupils, and subsequently employed by Hasselbach, Linhard, R. G. Pearce and others. The obvious limitations to investigations of this type have prompted some investigators to employ decerebrate animals, or those in which the medullary centers are kept alive by artificial perfusion. The objections to the latter type of observation are too well known to require further comment here; they may or they may not be such as to render the results inapplicable to the intact animal. The chief objection to the use of decerebrate animals lies in the fact that the reactivity of the isolated centers is uncertain. This is particularly so in the case of the respiratory center. Some animals retain for several hours after the decerebration, a uniform and regular respiratory rate and volume, whilst others show an abnormal type of breathing. These irregularities, apparent in the work of Porter, Means and Newburgh, were also observed in the animals used by my former associate, R. W. Scott, in whose experiments it was further noted that apart from the animals that failed to breathe properly from the start, there were others which were apparently perfectly normal in this regard for some time (1–2 hrs.) after the decerebration, but in which later the breathing became dyspneic and irregular, and death soon followed, usually after an acute attack of vomiting.

As a preliminary to an investigation into the nature of the respiratory hormone, it was considered essential to investigate the cause of this delayed dyspnoea of decerebrate animals, not alone because these are probably the most suitable for use in such

investigations, but also because the behavior of the abnormal animal strongly suggests the possibility that development of a condition of acidosis is responsible for the symptoms. Some of the most conspicuous of the results so far obtained are reported here.

CAT. No. XXII.

| Time after Decerebration. (Min.). | Respiration per Min. | | Alv.-$CO_2$ (Per Cent.). | Blood-$CO_2$ (Per Cent.). | Blood $P_H$. | Blood L.A. (Per Cent.). | Urine. | | Rect. Temp. °C. |
|---|---|---|---|---|---|---|---|---|---|
| | c.c. | Rate. | | | | | N to Acid (Per Cent.). | $NH_3$ (Per Cent.). | |
| 53 | 1125 | | | | | | | | |
| 70 | | | 3.5 | | | | | | |
| 73 | | | 3.6 | | | | | | |
| 78 | 1080 | 28 | | | | | | | 39 |
| 93 | | | | | | | 30 | | |
| 108 | 1225 | 28 | | | | | | | |
| 118[1] | | | 3.3 | | | | | | 40 |
| 133 | | 38 | | | | | | | |
| 138 | | | 2.9 | | | | | | |
| 148 | | | | | 7.4 | | | | |
| 161 | | 38 | | | | | | | |
| 171 | | | 3.0 | | | | | | 40 |
| 178 | | 44 | 1.6–1.8 | | | | | | |
| 203[2] | | | 1.7 | | | | | | |
| 208 | | | | 24.4 | 7.1 | 0.296 | 30 | | |

CAT. No. XXIII.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 0.107 | |
| 90 | | | | | | | 106 | 0.076 | 38.5 |
| 135 | 1080 | 27 | | | | | | | |
| 140 | | | 3.3 | | | | | | |
| 170 | | | | | | | | | |
| 195[3] | 1120 | 28 | | | | | | | |
| 210 | 1170 | 28 | | | | | | | |
| 215 | | | 3.3 | | | | | | |
| 230 | 1150 | 30 | | | | | 20 | 0.326 | |
| 250 | 1120 | 28 | | | | | | | |
| 255 | | | 2.8–3.0 | | | | | | |
| 285 | | | 2.9 | | | | | | |
| 290 | 950 | 26 | | | | | | | |
| 293 | | | 2.9 | | | | | | |
| 295 | 930 | 24 | | | | | | | |
| 302 | | | | | 7.6–7.7 | | | | |
| 304 | | | | 45.0 | | 0.098 | 6.5 | | |
| 305 | 960 | 22 | | | | 0.101 | | | |

The animals (cats) were decerebrated by the method of Miller and Sherrington. In those on which regular breathing returned, an interval of one hour was allowed to elapse, so that the

[1] Suddenly hyperpnœic.

[2] Vomited.

[3] Rigidity slight.

influence of the anesthetic (ether) might have ample time to disappear, and then observations were made on the following: (1) The minute volume of air breathed; (2) the alveolar $CO_2$; (3) the total $CO_2$, $P_H$ and (5) the lactic acid content of the arterial blood; and lastly, (6) the total acid excretion by the urine.

The general nature of the results is indicated in the following table in which the above values are given for an animal which showed no dyspnea (XXIII), and one in which this and irregular breathing were pronounced (XXII).

These experiments typify the results in extreme cases; the animal in XXIII remained in perfect condition for over five hours after the decerebration, whereas in that of XXII the breathing, although normal at the start, became later rapid and dyspneic, death, preceded by vomiting, occurring in about three and one half hours after the decerebration. Of a total of thirteen animals so far observed, six behaved like XXIII, for at least five hours, and four like XXII, while three gave intermediate results. Animals in both of the latter groups died within three hours. In the animals of the second group which provisionally we may call the acidosis group, the following changes were invariably found: (1) A progressive decrease in alveolar $CO_2$ followed later by (2) a decrease in blood $CO_2$, (3) an increase in acidity ($P_H$ lower and (4) an increase in the lactic acid content of the blood. The excretion of acids and ammonia by the urine was irregular. The simplest interpretation of the results is that the development of a condition of acidosis is responsible for the changes observed in the dyspneic group of animals. It is further of interest to record, that decerebrate rigidity was much more pronounced in the "acidosis" animals than in those that remained normal. Whether the rigidity is responsible for the acidosis, by causing lactic acid to be discharged in excessive quantities into the blood, or whether it is an effect of the acidosis, is at present problematical.

Marked glycosuria was common in most of the animals.

# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES


No. 23: THE DIAGNOSIS OF ACIDOSIS, BY PROFESSOR J. J. R. MACLEOD

(REPRINTED FROM THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, VOL. IV)

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# THE DIAGNOSIS OF ACIDOSIS

A Review and Criticism of the Methods at Present in Use

BY

J. J. R. MACLEOD, M.B.,
Toronto, Canada

Reprint from

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St. Louis

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# THE DIAGNOSIS OF ACIDOSIS*

## A Review and Criticism of the Methods at Present in Use

By J. J. R. Macleod, M.B., Toronto, Canada

THERE has been some confusion in medical literature concerning the exact meaning of the term "acidosis," so that it will be advantageous to preface our discussion of the subject with a brief review of the work upon which the present-day definition is based. The discoveries that large quantities of oxybutyric and oxyacetic acids are excreted in the urine of patients suffering from diabetic coma, and that there is a general similarity between the symptoms of this condition and those which follow the intravenous injection of strong acids (acid intoxication) in laboratory animals, were primarily responsible for the adoption of the term. In seeking earlier signs of acidosis than the actual symptoms of coma, however, examination of the urine for oxybutyric and acetoacetic acids or their oxidation product, acetone, was found to be of uncertain value, since these substances might also appear in decidedly large amounts in the urine of nondiabetic individuals. During starvation, for example, either complete or involving carbohydrate foods alone, acetonuria was frequently met with, which made it clear that the excretion of acetone bodies could not in itself be taken as a reliable indication of impending acidosis.

It was attempted therefore, to develop methods of diagnosis depending, not on the detection of the particular acids, but on the effects which might be produced by the accumulation in the organism of acids in general.

In the first place it was natural to expect that the blood would become less alkaline as the result of the acid production, and attempts were therefore made to measure the alkalinity of the blood by titrating with standard acid until the point of neutrality was reached, as judged by the change of tint of some indicator. It was hoped that the values obtained by this method would indicate in the blood of cases threatened with acidosis, the presence of more acids than in normal blood; that is, that a smaller amount of standard acid would require to be added to the former, than to the latter blood, in order to cause the tint of some indicator to change. Although with certain modifications this method is theoretically sound, it was found to have little practical

*Delivered before the Medical Section, Academy of Medicine, University of Toronto, Toronto, Canada.

value, partly because it requires large amounts of blood, and partly because the coloring matter has to be removed before the test can be applied. Moreover, it came to be recognized that the amount of added acid, with the indicators ordinarily used, would represent, not only the basic constituents that combine with acids in the quantities in which these could be produced in the organism, even under abnormal conditions, but also those, like proteins and phosphates, that might be called upon to functionate as bases when the limits of acidity compatible with life were greatly overstepped.

It became necessary to seek for some other type of reaction which acids might set up in blood, and the well-known effect which these have in expelling carbon dioxide from its combinations with alkalies was investigated (Walter). It was found by experiments on animals that a marked diminution in the $CO_2$ content of blood was induced by intravenous injections of acids, and the same was observed to be the case in the blood of patients in diabetic coma (Naunyn).[1] In the light of more modern research, by which, as we shall see later, the delicacy and accuracy of this method is fully justified, it is somewhat surprising that it did not receive more extensive application. One serious difficulty stood in the way, namely, the technic of the estimation.

The next step depended on the discoveries of Haldane and Priestley[2] that the percentage of $CO_2$ in the alveolar air of normal individuals at atmospheric pressure is remarkably constant, and of Krogh that the amount of free $CO_2$ in the arterial blood is very nearly the same as that in the alveolar air. These facts led Haldane and his collaborators to formulate the hypothesis that the alveolar $CO_2$ must be proportional to the relative amounts of carbonic and of other (fixed) acids in the blood, and that when the latter are increased there must be a compensatory decrease in the former, provided the control of the respiratory function is normal. The first application of this principle on pathologic acidosis was made by Beddard, Pembrey and Spriggs,[3] who examined the alveolar $CO_2$ in diabetic patients, with the result that a very pronounced diminution was found whenever the comatous condition existed or was threatened. The rationale of this method and its limitations we shall discuss later; for the present it may be stated that the alveolar $CO_2$ may also be depressed in other diseases, particularly nephritis, as well as in normal persons whenever acids accumulate in the organism as a result of deficient oxidation in the tissues (Haldane, etc.).

Clearly, therefore, the term acidosis must not be confined to cases of diabetic coma. On the other hand the common appearance of acidosis in this disease, coupled with the fact that in it the acids are of a type that is different from that found in nephritis, or during partial asphyxiation, makes it important that some term should be set aside to designate the diabetic condition. Since the acids are closely related to ketones (acetones) in chemical structure, the term "ketosis" has been suggested, and is gradually coming into general use. Ketosis, therefore, means the form of acidosis that is caused by the appearance of ketonic acids in the organism. Other forms of acidosis have not as yet been dignified by special names.

The remarkably rapid development of our knowledge of this subject during recent years, coupled with its dependence upon certain fundamental principles of physical chemistry, has made it very difficult for those of the medical profession who have been out of college for several years to appreciate the full significance and value of closely observing the neutrality regulation in the animal body in order that incipient states of acidosis may be detected. I will therefore attempt to review very briefly these fundamental principles, and to explain the various tests by which alterations in the reaction of fluids in general may be detected.

In the first place we must clearly understand what is meant by an acid such as HCl, and an alkali, or base, such as NaOH. When the molecule of either of these chemicals is dissolved in water it splits, or dissociates, into two portions called ions; in the case of HCl, for example, ions of H and Cl are formed, and in the case of NaOH, Na and OH. Moreover, each of the ions carries an electric charge, and this is always of opposite sign for the two ions composing the molecule. The sign of the electric charge for the H ion and all metals is positive, and that of Cl and all acid groups is negative. Since, as is well known, unlike electricities attract each other, this means that the positive ions like H would be attracted towards the negative pole or cathode of a pair of electrical terminals, between which a current is passing, placed in the solution. They are, therefore, called cations, and for the same reason the negative ions are called anions. To indicate these electrical charges, a dot is used for positive, and a dash for negative, thus, $H^{\cdot}$ and $Cl'$.

But what, it may be asked, have these principles of electrolysis to do with the question of reaction? The answer is that it is the presence of free $H^{\cdot}$ ions that determines the acidity of a solution. If we examine the formula of all known acids it will be seen that they have hydrogen in some displaceable or dissociable form in the molecule, and, furthermore, if we measure the acidity, as judged by the common standards of this property, such as the ability to dissolve metals, the acid taste, the power of inverting sugars and starches, and so forth, we shall find that it runs parallel with the dissociability of the H ion from the rest of the acid molecule. The $H^{\cdot}$ ions present in a free state in the solution must, therefore, be an accurate measure of its acidity. This leads us to expect that the presence of free $OH'$ ions must be a measure of alkalinity, since we know that when an acid and a base are brought together, the one neutralizes the other by a reaction which consists essentially in the combination $H^{\cdot}$ and $OH'$ ions to form $H_2O$. This removes the ions from the free state and so locks them up, because molecules of water practically do not dissociate.

We have learned two fundamental principles, namely, that the standard of perfect neutrality must be where $H^{\cdot}$ and $OH'$ ions exactly balance each other, and that the true acidity of a solution will be represented by the excess of free $H^{\cdot}$ ions over $OH'$ ions, that is, by the $H^{\cdot}$-ion concentration ($C_H$).

But we can go further, for not only may the acidity be expressed in terms of $C_H$, but so also may the alkalinity. Why should this be? It is clear that the most strictly neutral solution must be pure water, in which H· and OH′ ions are exactly counterbalanced. Nearly all of the H· and OH′ are combined in an undissociated molecule $H_2O$, but not all, for even in the purest water a slight degree of dissociation occurs, giving us therefore H· and OH′ ions. When the concentrations of the two ions are multiplied together the product is $1.2\times10^{-14}$, which means that there are 1.2 gram molecules of hydrogen (or its equivalent) present in, 10,000,000,000,000 liters. Since the concentrations of H· and OH′ ions are equal, the H·ion must therefore, be $1.2\times10^{-7}$, which means that this ion is present so as to form a .000,000,12 N solution is 1.2 gm. H· in 10,000,000 liters.

When some acid is added to the water the con. of H·ions, of course, rises, but, and this is the fundamental point to bear in mind, the con. of OH′ ions correspondingly falls so that, as in pure water, *the product of the two concentrations is again* $1.2\times10^{-14}$. However acid or alkaline a solution may be, the product of the concentrations of the H· and OH′ ions is always the same. Clearly then *we may express the reaction even of alkaline solutions in terms of the H ion concentration.* Whenever this is greater than $1.2\times10^{-7}$, the reaction is acid, but when it is less than $1.2\times10^{-7}$, the reaction is alkaline.* It is usual to abbreviate the expression hydrogen-ion concentration into $C_H$.†

These considerations lead us to seek for methods by which $C_H$ may be measured. These are two in number, namely, the electrical and colorimetric. Concerning the former, suffice it to say that it consists in measuring the voltage or electric force set up in a battery of which one electrode is pure hydrogen gas in intimate contact with the solution whose $C_H$ we desire to measure, and the other electrode is one of known voltage, such as the so-called calomel electrode. The rate of diffusion between the free H· ions in the solution and the H which constitutes the one electrode, is naturally dependent upon the concentration of free H· ions, and it is on this that the development of electric force depends at this electrode. Consequently since everything else is constant in the battery, the total electromotive force must be proportional to the $C_H$ of the unknown solution.

The colorimetric method is much simpler, but not so delicate. It depends on the fact that certain indicators change in tint in proportion to $C_H$. In titrating solutions, such as the stomach contents, for the degree of acidity, the impression is apt to be formed that the tint changes suddenly at the neutral point. This is not the case however, for if the titration be done by very small additions of acid or alkali about the neutral point, it will be found that there is a fine gradation from the typical acid color to the typical alkaline color.

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*The exact value of 1.2 varies with temperature. When $C_H$ becomes greater than that of neutrality the number 1.2 becomes greater, and if the change be beyond 10 the characteristic (-7) becomes less.

†For convenience of expression it is usual to designate the H-ion concentration by $P_H$ instead of $C_H$. $P_H$ is obtained by finding the logarithm of the number of gram molecules of H (i.e., 1.2 in the above example), and subtracting this from the characteristic (i.e.,-7 in the above example). Since the minus sign is understood $P_H$ increases in magnitude as the H-ion concentration (expressed by $C_H$) decreases. In the present article $C_H$ is used because its use is less confusing in a general discussion of the acidosis problem.

The exact $C_H$ at which indicators change in tint varies with the indicator. Thus phenolphthalein, which is used in certain titrations of stomach contents, changes in tint at a much lower $C_H$ than methyl orange or litmus, and with none of these indicators does the point of change occur at a $C_H$ of $1.2\times10^{-7}$, so that they are unsuitable for measurement of the $C_H$ of solutions that are nearly neutral. Fortunately, however, such indicators exist, the best known of which are sulphophenolphthalein, rosolic acid, and neutral red. If therefore we take a series of solutions having slightly variable, but known, $C_H$ about the neutral point, and add to each a drop of one of the latter indicators, we shall obtain a series of graded tints, and the tint will be proportional to the $C_H$. The preparation of the standard solutions is not a difficult matter, provided ordinary precautions are taken. Mixtures of acid and basic phosphates in varying proportions are most practical.[4] The resulting mixtures should be measured electrometrically for accurate work. In order to determine the $C_H$ of an unknown solution, some of it is placed in a hand glass test tube and an amount of suitable indicator is added so that the proportion of indicator to solution is the same as in the standards. The tint of the standards with which the tint of the unknown matches is then ascertained and this gives $C_H$. When the colorimetric method is employed for blood, it is of course necessary to get rid of the blood pigment and also of the proteins since these interfere with the reaction. This is accomplished by placing a few cubic centimeters of blood in a dialyser in the shape of a collodion tube, and suspending this in neutral physiologic saline. The saline soon assumes the same $C_H$ as the blood, and this can be measured by the above described method.

When $C_H$ of the blood is measured by one or other of these methods the significant fact is revealed that it is practically always the same, and is not far removed from that of pure water; at 38° C. $C_H$ equals $0.4\times10^{-7}$. Even in severe cases of diabetic ketosis, an increase in $C_H$ becomes perceptible only in the final stages of the condition. Clearly, therefore, even the slightest increase in $C_H$ is incompatible with life, and $C_H$ of the blood must be considered as a physiologic constant. It is greatly more so than body temperature or blood pressure, so that its measurement can be of little practical value in the clinic, and the question arises as to how the early stages of changes that might ultimately end in death from an increase in $C_H$ may be detected. To answer this question we must study *the nature of the mechanism by which neutrality is maintained in the organism;* in other words why it should be the case that large quantities of acid can be produced in the body, as in diabetes, without any perceptible change in $C_H$.

When even a trace of acid is added to water or an isotonic solution of sodium chloride, a very pronounced change occurs in $C_H$, but it requires ever so much more acid to produce any perceptible change in the case of blood. For example, using the colorimetric method let us observe the change in tint produced by adding a drop of weak HCl to water containing sulphophenolphthalein and then see how much of the same acid must be added to bring about a similar

change of tint in a dialysate of blood. It takes very much more. Clearly the blood contains something which as it were soaks up the added H ions. This has been called the *buffer action* of blood, or better still, the *tampon action.* The question is, to what is this buffer action due? A clue is furnished by using solutions of phosphates. For example, if we take a solution containing alkaline and acid phosphates in such proportion that $C_H$ is the same as that of blood, and then add acid, we shall find that ever so much more has to be added than is the case of water. The same is true of solutions of bicarbonate.

This property of phosphate has been carefully studied by L. J. Henderson,[5] to whose brilliant researches we are primarily indebted for the recent development of our knowledge in this whole question. Henderson found that weak acids like acid phosphate and carbonic acid possess the property of holding the H-ion concentration nearly constant, i.e., of acting as buffers, when they are present in solutions containing an excess of their salts. Now, in so far as the blood plasma is concerned, it is not phosphates, but rather bicarbonates to which the buffer action must be due, and as a matter of fact it has been found that $C_H$ is directly proportional to the ratio existing between $CO_2$ in solution as $H_2CO_3$ and sodium bicarbonate $NaHCO_3$ multiplied by a constant; or, expressed in chemical notation, $C_H$ = the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$. This ratio is $\frac{1}{20}$, and we may define acidosis as any condition in which the proportion between $H_2CO_3$ and $NaHCO_3$ becomes greater than 1:20. It must be clearly understood that this applies only to isolated plasma, for when whole blood is used, other substances come into play in maintaining neutrality; the phosphates, for example, though practically absent from plasma, are nevertheless present in the corpuscles through the envelopes of which diffusion more or less readily occurs, so that they serve as a reserve buffer. Proteins also may serve either as weak acids or alkalies, and therefore neutralize quite decided quantities of added acid or alkalies. But for practical purposes we may regard the buffer agency as being the ratio $\frac{H_2CO_3}{NaHCO_3}$ and the problem of finding practical means for the detection of threatened acidosis now narrows itself down to a study of the behavior of this ratio.

Suppose that some fixed acid were added to a buffer solution containing bicarbonate. It would react with $NaHCO_3$ to form a neutral salt of the acid, and consequently set free some $H_2CO_3$, that is, it would diminish the denominator but increase the numerator of the equation $\frac{1}{20}$. The increase in $C_H$ would not be proportional to that of the added acid, on account of the buffer mechanism (because $H_2CO_3$ does not dissociate well) but it would nevertheless increase somewhat just as any kind of buffer takes up most, but not all, of a force applied to it. This indicates that if the foreign acid were being added continuously, as would be more or less the case in pathologic acidosis, $C_H$ would rise in spite of the buffer unless some method existed for decreasing the numerator of the equation, i.e., getting rid of $H_2CO_3$. This is one of the functions of the lungs, the $CO_2$ excretion through which must therefore, be considered as a

necessary part of the neutrality mechanism of the body. The $CO_2$ is got rid of until the ratio $\frac{H_2CO_3}{NaHCO_3}$ comes back to its old level of $\frac{1}{20}$. But now, much of the $NaHCO_3$ having been used up to combine with the foreign acid, the actual amount present is much less than before. This means that the $H_2CO_3$ must also be less, that is, the $CO_2$ in a free state in the blood plasma. Now, since as we have seen, the pulmonary epithelium permits the free $CO_2$ of the blood to diffuse readily through it, it follows *that the percentage of $CO_2$ in the alveolar air must be a measure of the available $NaHCO_3$ in the blood.* To repeat, for this is the fundamental conception of the whole acidosis problem, since $C_H$ remains constant in the blood the ratio $\frac{H_2CO_3}{NaHCO_3}$ must also remain at its normal value of $\frac{1}{20}$, and, therefore, if $NaHCO_3$ declines, $H_2CO_3$ must decline proportionately, and since this diffuses as $CO_2$ into the alveolar air, the percentage of this gas in the latter must be proportional to the degree to which foreign acid can be added to the blood without perceptibly changing $C_H$, in other words it must be proportional to the reserve alkalinity.

One other factor must clearly come into play to permit of the smooth operation of the above mechanism, namely, the rate of pulmonary ventilation must be adapted to the amount of $CO_2$ that has to be eliminated. This adaptation depends on the respiratory center, the activity of which is preeminently dependent upon the acid base equilibrium in the blood.

It is commonly taught that the thing that really stimulates the respiratory center is not the $CO_2$ itself, but the slight increase in $C_H$ which, as explained above, inevitably occurs, in spite of the buffer action. This is, however, probably an incorrect view, for R. W. Scott,[6] working in my laboratory, has found that $CO_2$ itself can excite the center quite independently of the H-ion concentration of the blood. Thus Scott found after injecting sodium carbonate into decerebrate cats until the $C_H$ was very decidedly lowered, that a subsequent increase in the free $CO_2$ of the blood excited respiration almost to the same degree as would have been the case when the $C_H$ of the blood was the normal, and furthermore, that when this excitement occurred, the blood was still markedly alkaline.

So long then as the center responds immediately to the slight excess of free $CO_2$ this is got rid of, and the normal ratio between $H_2CO_3$ and $NaHCO_3$ is reestablished. If the sensitivity of the center should be below par, however, then $CO_2$ might accumulate in the body and $C_H$ consequently rise. As a matter of fact, even in health, it seems to be established that the excitability of the center may vary, as for example, in relationship to the amount of oxygen in the blood supplying it, and in disease there can be no doubt that such variations in excitability occur. And since we know that the activities of this center are also greatly affected by nerve stimuli, arriving at it along afferent nerves, it is to be expected that it may be keyed up or down in excitability by the state of the nervous system in general. In conditions of nervous excitement and anxiety, for example, its sensitivity is increased and moderate doses of narcotic drugs, such as morphine, are well known to depress it.

But even were the sensitivity of the center maintained at a constant level, the alveolar-$CO_2$ would correspond with that of the arterial blood only provided that the exchange of $CO_2$ across the alveolar epithelium was strictly normal. And when we bear in mind that the proper excretion of the $CO_2$ in the lungs is dependent upon an accurate adjustment between the heart's action and the rate of $CO_2$ production in the organism, we see how the mechanism might readily become upset.

Technical difficulties have also to be overcome in the collecting of the alveolar air, for it is now well established that the original method of Haldane and Priestley is approximately accurate only when it is carried out under strictly controlled conditions—so strict that they can not be practiced in the clinic—and even then, as R. G. Pearce, Carter, Krogh, Siebeck and others[7] have shown, we can not be certain of the results. At best, therefore, *the alveolar $CO_2$ can serve as an accurate index of the acid base equilibrium of the blood only under certain controlled conditions.*

These facts have prompted the most recent observers (Morawitz and Walker[8] and later Van Slyke and Cullen[9]) to return to blood examination for the detection of impending acidosis. The question is what readily measurable property of the blood may we employ? If we return for a moment to the equation $\frac{H_2CO_3}{NaHCO_2} = \frac{1}{20}$ we shall see that when foreign acids combine with the Na of the bicarbonate, this will become replaced by the salts that are formed, such as NaCl, and these will be incapable of acting as buffers. The amount of $NaHCO_3$ present in the blood must, therefore, be a measure of its power to take up such acids without serious disturbance in $C_H$, which has led Van Slyke to define acidosis as "a condition in which the concentration of bicarbonate in the blood is reduced below the normal level." According to this definition, acidosis is not necessarily an increase in the actual $C_H$ of the blood, or even it is a disturbance in the normal ratio $\frac{H_2CO_3}{NaCO} = \frac{1}{20}$ but is a lowering of the absolute values forming the numerator and denominator of the equation. In brief, it is a lowering of the ability of the blood to take up fixed acid without disturbance in the ratio, a decrease in the reserve alkalinity. The definition is, however, somewhat unfortunate, since it does not include cases in which there is an actual increase in the ratio as a result of the addition of $H_2CO_3$ to the blood, and such conditions may develop as a result of asphyxia, which, of course, is a common enough cause of acidosis in the broader sense. Moreover, in asphyxial acidosis it may quite well be the case that the $NaHCO_3$ instead of being diminished, is actually increased in the attempt to bring the ratio back to its normal value. When $CO_2$ is added to blood, for example, it has been shown that the alkali content of the plasma relatively increases, partly because of the migration of K and Na out of the corpuscles into the plasma, and partly because HCl goes in the opposite direction. There can be no doubt, however, that deficiency in bicarbonate is an im-

portant thing to measure as a gauge of the ability of the blood plasma to hold $C_H$ constant when foreign acids are added to it.

In its newer form this test differs considerably from the form in which it was originally employed by Walter (see p. 3), for instead of measuring the $CO_2$ content of a sample of blood immediately after removal, this is first of all exposed outside the body to an atmosphere containing a known, fixed, percentage of $CO_2$ until all of the available alkali has become combined with this gas. This procedure removes the most serious objection to the old technic, namely, that the amount of $CO_2$ in the venous blood,—and that alone is of course available for use in man,—must be very largely dependent upon the rate of oxidation in the tissues, and upon the velocity of movement of blood through the capillaries which drain into the vein. In experimental work on animals, however, the $CO_2$ actually present in arterial blood remains as the most practical indicator of the buffer action of the blood. Being restricted in man to the use of venous blood, the questions which remain to be answered are: 1. What pressure of $CO_2$ should be chosen with which to saturate the blood?* 2. Should whole blood or plasma or serum be employed? With regard to the $CO_2$ pressure, there are two alternatives: we may use either the pressure to which the blood is actually exposed in the organism, the *intra vitam* pressure we may call it, or an arbitrarily fixed pressure. The *intra vitam* pressure of $CO_2$ can be determined by analysis of the alveolar air, but this, of course, complicates the technic and gives us no more accurate an estimate of the available alkali than when we use the same pressure for all samples.

Concerning the question as to whether whole blood or serum should be used, there can be little doubt that it should be the former. The objection to the use of serum or plasma depends on the fact, as we have seen, that a part of the alkaline reserve resides, not in the fluid menstruum of the blood, but in the corpuscles, and even beyond this, in the tissue cells. It has been known for some time, for example, that when $CO_2$ is bubbled through whole blood, the alkali content of the plasma decidedly increases, as judged by titration, and when the blood is in contact with the tissue cells it is quite likely that when excess of acid appears in it, there may be a considerable transference, not only of more alkali from the cells into the blood, but also of $CO_2$ in the opposite direction, (into the cells including the red blood corpuscles) in which it may become combined with alkaline phosphates by such reactions as are illustrated in the equation:

$$Na_2HPO_4 + H_2CO_3 = NaH_2PO_4 + NaHCO_3.$$

It should be remarked that Van Slyke recognizes these possibilities, but nevertheless, he believes that for practical purposes it is allowable to employ venous blood, and to saturate its plasma with $CO_2$ at a definite partial pressure. The results which he and other workers using his method have employed would certainly appear to justify his claim at least for the detection of cases in which the alkaline reserve is decidedly reduced, but it should not be lost sight of that a strict observance of the principles which we have attempted to explain might

*It may be pointed out, for the sake of those who are not familiar with the work in this field, that the pressure of a gas in a mixture of gases is proportional to its percentage amount; thus 6 per cent $CO_2$ will give 45.6 mm. Hg pressure at a barometric pressure of 760, for 100 : 760 : : c : x.

make the gauging of the alkaline reserve a still more valuable criterion of disturbances in the acid base equilibrium in the organism.

To sum up, it may be said that determination of the ability of blood to absorb $CO_2$ is probably the most practical method for measuring the acid neutralizing power; it measures the degree to which the acid buffer can functionate or, as some call it, the alkaline reserve. This high estimate of its value holds good, however, only when the whole blood is taken, but *even then we do not necessarily measure the total reserve of the body.* These reserves are, first, the alkalies of the plasma, second, the alkalies of the corpuscles, third, the proteins of the blood, and the last reserves are probably the alkalies and the proteins of the tissue cells. Now it is clear that there can be no test-tube method by which the magnitude of all of these defensive agencies could be measured; at best we can only measure the first three of them, namely, the reserve power resident in the blood itself. Christiansen, Haldane and Douglas[10] and contemporaneously Morawitz and Walker,[8] have recommended this method, and it is only more recently that Van Slyke, in order to simplify the technic, has advocated the employment of blood plasma (oxalated) alone. Let us consider for a moment, therefore, whether the Van Slyke technic is really much simpler than that employed by Haldane. In the method used by these observers a cubic centimeter, or so, of defibrinated blood is exposed at body temperature for twenty minutes, in an air-tight vessel, to an atmosphere containing a known amount of $CO_2$. A measured sample is then removed to the gas analysis apparatus of Haldane-Barcroft, and the $CO_2$ is determined by decomposing the carbonates with strong acid, after getting rid of the oxygen by shaking with ferricyanide solution. The procedure is comparatively simple, and can easily be done with a little practice in the wards.

In Van Slyke's method, the oxalated plasma, separated by rapid centrifuging, is exposed to an atmosphere of expired air in a tonometer, and the plasma then transferred to a gas analysis apparatus, that is certainly no simpler in manipulation than that of Haldane and Barcroft (see Vol. II, p. 55 of this Journal), and which moreover suffers from the disadvantages: first, that the slightest leakages around the stopcock involves a very serious error because of the vacuum which is established in the apparatus at a certain stage of the manipulation, and secondly, that it requires the use of mercury which becomes fouled with the mixture of plasma and acid during the analysis.

Taking into consideration the theoretic objections to the use of plasma or serum in place of blood, and also the doubtful advantage to be gained by using the Van Slyke apparatus, at least by those who are not practiced in the use of gas analysis methods in general, there is no very evident reason why the Van Slyke procedure should be followed in preference to the earlier, really simpler methods of Haldane, etc., and of Morawitz and Walker.

It has been pointed out above that the sensitivity of the respiratory center towards the $C_H$, or more probably the free $CO_2$, of the blood is apparently much greater than that of any other center or mechanism in the animal body, and that it is on account of this sensitiveness that the free $CO_2$ of the blood is immediately got rid of from the body by increased pulmonary ventilation, whenever there is tendency for the $C_H$ to rise above the normal level. As the free $CO_2$ is got rid of,

the bicarbonate decomposes, because of the presence of other acid groups in the blood, e.g., protein, etc., and the amount that is left indicates the remaining ability of the blood to withstand further addition of foreign acid. Clearly, therefore, the important thing to measure in order that we may be enabled to diagnose the incipient stages of acidosis is the alkaline reserve, and but one question remains to be considered, namely, whether arterial or venous blood should be employed. For various reasons arterial blood is preferable. In the first place the percentage of $CO_2$ actually present in it is proportional to the alkaline reserve, so that it is unnecessary to expose the blood to an atmosphere containing $CO_2$ before measuring the $CO_2$ content, and in the second place, it represents the mixed blood of the body, and not that of only one locality, as is the case with blood removed from a peripheral vein. But in clinical practice venous blood only is available. If this is collected with the precaution that the muscles in the corresponding area have been at rest for some time it appears that there is practically no difference between the alkaline reserve of arterial and venous blood, but if there has been any muscular contraction, the venous blood will have a lower reserve than the arterial, because of the lactic acid thrown into it by the contraction. But even when we take the precaution of avoiding muscular action, it is probable that there is not a strict parallelism between the buffer action of arterial and venous blood as in cases in which the demands on the alkaline reserves are such that those of the tissues are being called on as well as those of the blood itself. The experiment of Van Slyke,[9a] in which he compared actual $CO_2$-containing powers of arterial and venous blood removed from anesthetized dogs, and found them to be very nearly the same, does not throw any light on this phase of the problem.

The chief criticism against the use of the $CO_2$ carrying power of blood or blood plasma, is therefore, that it tells little if anything concerning the acid-absorbing powers of the tissues. Is there not, therefore, some test of the acid buffer which can be applied to the intact animal? One such we have already considered, namely, the percentage of $CO_2$ in alveolar air, and we have seen that it is not entirely satisfactory partly because of the technical difficulties in the collection of the sample of air for analysis, and partly because of possible variations in the sensitivity of the respiratory center. In order to place an estimate on the relative value of these methods comparisons have been made between the $CO_2$ tension of the alveolar air and the $CO_2$ absorbing power of the blood. This has been done both in normal and pathologic subjects. In normal subjects the comparisons have been made under conditions, such as the taking of food and during muscular exercise, in which slight alterations in the acid-base equilibrium are known to occur. Van Slyke, Stillman and Cullen[9b] found that the ratio $\frac{\text{plasma } CO_2}{\text{mm. alveolar } CO_2}$ varies from 1.27 to 1.80 in different resting individuals, there being apparently a characteristic ratio for each individual, and that the taking of food invariably raises the alveolar $CO_2$-combining power. This would seem to indicate that it must be the excitability of the respiratory center rather than the acid base equilibrium that becomes altered so as to cause variations in alveolar $CO_2$. The same authors, in conjunction with Fitz, working on the above

relationship in patients suffering from diabetes and nephritis, found that in the former disease, under treatment, the alveolar $CO_2$ tension is often much too low in comparison with the blood bicarbonate. On the other hand the tension is always low in these cases when the bicarbonate content of the blood is low. In nephritis, however, the tension of alveolar $CO_2$ may be high although the blood $CO_2$ is low. Peters[12] has also examined these relationships, and has met with many instances where satisfactory parallelism did not exist. One serious criticism of this work is, however, that the method of Fridericia was employed for collecting the alveolar air.

Christiansen, Douglas and Haldane[10] did not find a relation to exist between the $CO_2$ absorbing power of the blood and the normal resting alveolar $CO_2$ in different healthy individuals, but after severe muscular exercise, the interesting discovery was made that marked reductions occurred both in the $CO_2$ absorbing power of the blood, and in the alveolar air, and they suggested "that corresponding differences will probably be discovered under various pathologic or compensatory conditions for acidosis."

In all these comparisons it is with the arterial $CO_2$ tension that the alveolar $CO_2$ is believed to run parallel, the air samples being removed from the alveoli under conditions which mean that the gaseous equilibrium must be between alveolar air and arterial blood. The question therefore arises whether more exact parallelism between alveolar $CO_2$ and blood $CO_2$ might not be obtained if the venous $CO_2$ tension were taken instead of the arterial. As shown by Christiansen, Douglas and Haldane, and later by Y. Henderson[11] and R. G. Pearce,[13] the alveolar venous $CO_2$ tension can be measured by the comparatively simple expedient of causing the patient to inspire an atmosphere containing a percentage of $CO_2$ above that which corresponds to the free $CO_2$ of venous blood, and then removing successive samples of the expiration which follows, and analysing each for $CO_2$. The portions expired at first will contain approximately the same percentage of $CO_2$ as that in the inspired air, but the percentages will progressively decline in the succeeding portions until at last they become constant at a level which must correspond to the venous $CO_2$ tension. In the method of Plesch for the collection of alveolar air, in which the person takes several breaths in and out of a rubber bag, the $CO_2$ percentage of the air in the bag must approximate more closely the $CO_2$ tension of the venous blood than that of the arterial. It is certainly important that the possibility of a parallelism between alveolar venous $CO_2$ tension and $CO_2$ combining power of the whole blood should be thoroughly investigated. On *a priori* grounds the parallelism is likely to be a much closer one than when the comparison is made between alveolar (arterial) $CO_2$ tension and $CO_2$ combining power of blood plasma.

By an application of these principles, therefore, it does not appear that there is a simple, thoroughly reliable method by which the buffer action of the body as a whole can be measured. An entirely different plan of attacking the problem must be thought of, namely, to observe the acid excretion by way of the urine.

When we were considering the general nature of the chemical reactions between the salts of the blood and added acid we saw that besides free $CO_2$ there

would have to be eliminated from the body a considerable amount of acid salts, or of free acids. In this connection the body must be considered as a whole, and when foreign acid is added a corresponding amount must ultimately be eliminated, if the normal acid-base equilibrium is to be reestablished, even although a sudden change in $C_H$ is avoided by the buffer action. Most of this excess of acid, as we have seen, is eliminated by the lungs as $CO_2$, but the remainder has to be got rid of by other pathways of excretion, particularly the kidneys. The excretion of the acid excess by this pathway occurs in three forms: *first* as acid salts, for example by conversion of $Na_2HPO_4$ into $NaH_2PO_4$, *second* as salts of ammonia and *thirdly* as free acid. Concerning the second of these, under normal conditions ammonia is a product of metabolism split off during the break down of amino acids, and then rendered innoxious, by combining with carbonic acid to form ammonium carbonate which is converted into urea, a strictly neutral substance. When excess of acids must be neutralized, however, some of the ammonia instead of combining with $CO_2$, may be diverted to neutralize the excess, this being particularly the case when ketonic acids appear, as in diabetes. The ammonia excretion itself is, however, no reliable indicator of the amount of free acid in the body, because there are other conditions such as derangement of the hepatic function which may influence it. In the toxic states of pregnancy, for example a large excretion of ammonia may occur without any indication of an acidosis. With regard to the acid which may be excreted in a free state, this occurs, for example, with $\beta$-oxybutyric acid, which is so weak in its acid properties, that 45 per cent of it may be excreted in this form.

With such a multiplicity of forms of excretion it is clear that *the only satisfactory method for measuring the acid excretion by the kidney must be one in which all of the above forms of excretion are included.* To measure the amount of free acids and acid salts the theoretically correct method would be to titrate the urine with standard alkali until its $C_H$, which is normally on the acid side of neutrality, was brought to the same level as that of blood. This can be done by using sulphophenolphthalein which, it will be remembered, changes tint at about the $C_H$ of blood. For practical purposes, however, it has been found more convenient to use phenolphthalein, the neutral point of which is such that when urine just reacts neutral to it, the $CO_2$ combining power of the blood plasma as tested by Van Slyke's method, is at its maximum by 80 vols. per cent, and the ammonia excretion is zero. By titrating a sample of urine after adding neutral oxalate to it,—in order to precipitate substances which interfere with the sharpness of the end point—with $N/_{10}$ NaOH to the neutral point of phenolphthalein we measure the total of free acids and acid salts, and if we now add to this the amount of ammonia in the same urine, (readily measured by Folin's new permutit method), we obtain the *total urinary acid excretion.* It is said that a definite relationship exists between the $CO_2$ combining power of the plasma and the total acid excretion, a relationship, however, which can be made clear only when a rather arbitrary equation like that of Ambard is employed. It remains to be seen of what value the determination of this ratio may be in clinical diagnosis.

There remains possible another method for gauging the alkaline reserve, namely, to see *how much alkali can be added to the organism without causing the*

*urine to assume an alkaline reaction.* When the alkaline reserve of the body is about normal, it is clear that very little alkali will suffice to have this effect. As a matter of fact, it has been found by Sellards[14] and by Palmer and Henderson[15] that only 5 grams a day, can be taken without making the urine alkaline. When the alkaline reserve is seriously depleted, however, large quantities of bicarbonate, even as much as 100 grams a day can be taken without making the urine alkaline. This test has been found of particular value in the diagnosis of acidosis accompanying certain forms of renal disease (chronic interstitial nephritis), which raises the question as to whether the retention may not be due to faulty elimination of the bicarbonate rather than to its retention in order that a deficient alkaline reserve may be corrected. It has not been a very simple matter to entirely disprove this possible explanation, and experiments of a variety of types have had to be devised in connection with the problem. One of them consists in determining the effect of a second dose of bicarbonate administered to an acidosis patient to whom a sufficient amount had previously been given to render the urine just alkaline. It has been found that a few grams now suffice, indicating, apparently, that the alkaline reserve must have been restored to its normal level. Even to this experiment the objection can be raised, however, that the large doses were retained because the threshold of the kidney for the excretion of bicarbonate was a very high one, and that the second, smaller administration just sufficed to overstep this threshold.

Sellards' careful work with this method seems quite clearly to establish its value, however, and for practical purposes *it is no doubt the best test of acidosis at present available in routine clinical work.* It has the important advantage, furthermore, of being simple and of requiring no elaborate apparatus.

Finally it may be advantageous to classify the possible causes which might lead to a want of stability in the $C_H$ of the blood; that is, to threatened acidosis or alkalosis, not of acidosis in the narrow sense implied in Van Slyke's definition, but in the broader sense of any disturbance in the acid-base equilibrium.

In general a tendency to acidosis might be due to an increase in the numerator or decrease in the denominator of the molecular equation $\frac{H_2CO_3}{NaHCO_3} = \frac{1}{20}$, or to a proportionate decrease in both. In the latter case, there would be no actual change in $C_H$, but the alkaline buffer would be depleted so that the change would very readily set in when foreign acids were added. Furthermore, it should be understood that $NaHCO_3$ only stands as a symbol for all substances that might serve as alkaline reserves, for although this salt is no doubt the most important of these, the alkaline phosphates of the corpuscles, and the protein of the blood and tissues must also be considered. A tendency to alkalosis—which is no doubt extremely rare as a pathologic condition—would be due to changes of a reverse character. A theoretic classification of the conditions which might cause these changes is given:

*Increase of* $C_H$.

| | |
|---|---|
| *Addition or accumulation of acid* | Accumulation of $CO_2$ (asphyxial conditions). |
| | Incomplete oxidation of carbohydrate (lactic and in muscular exercise). |
| | Defective oxidation of fat (ketosis). |
| | Renal insufficiency (nephritis). |
| | Decomposition of protein (as in acidosis of fever). |
| | Intestinal fermentation. |
| | Administration of acid (experimental). |
| *Decrease of base* | Diarrhea and hemorrhage, respectively (may explain acidosis in cholera and in certain forms of shock). |

*Decrease in* $C_H$.

| | |
|---|---|
| *Addition or accumulation of base* | Ammonia (faulty metabolism of urea). |
| | Intestinal putrefaction (infantile conditions). |
| | Administration of alkalies (experimental). |
| *Removal of acids* | Excretion of $CO_2$ (excessive pulmonary ventilation, as in faulty ether administration). |
| | Excretion of acid urine. |

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# SIMPLIFIED GAS ANALYSIS

No. III. The Dissociation Curve for Oxygen and the $Co_2$-combining Power of Blood

BY

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and
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# SIMPLIFIED GAS ANALYSIS

## NO. III. THE DISSOCIATION CURVE FOR OXYGEN AND THE $CO_2$-COMBINING POWER OF BLOOD

By J. J. R. Macleod, M.B., and R. S. Lang, B.A., Toronto, Canada

FOR accurate determination of the relative amounts of reduced and oxyhemoglobin in blood exposed to atmospheres containing varying partial pressures of oxygen no method surpasses that of Barcroft and his coworkers.[1]

The principle of this method is to expose a small quantity of blood in a thin film on the walls of a relatively large cylindrical vessel (tonometer) containing a mixture of nitrogen and oxygen gases until equilibrium has become established between the partial pressure of the oxygen in the atmosphere and the absorption of oxygen by the blood. Some of the blood is then transferred to a bottle connected with a differential manometer and shaken with dilute ammonia water, by which it becomes laked and takes up its maximal load of oxygen, thereby causing shrinkage in the volume of air, the degree of which is indicated by the manometer. The oxygen-saturated blood is then shaken with ferricyanide of potassium which dislodges the oxygen and causes the pressure in the bottle to rise. From the displacement of the fluid in the manometer in the two observations, the percentage of saturation of the blood with oxygen is readily calculated.

For use by a class of students the method is not practical because of the difficulty of providing suitable mixtures of oxygen and nitrogen with which to fill the tonometer and because of the expense of the differential manometer. The first of these difficulties is overcome by exposing the blood to a partial vacuum instead of a mixture of gases, a principle which has also been applied by W. G. Macallum, who uses a modified Van Slyke pipette both as tonometer and analysis apparatus.[2] The advantage of a partial vacuum over a mixture of oxygen and nitrogen is that the partial pressure of oxygen is readily calculated from the degree to which the tonometer is evacuated as measured by a barometer. In the present method the blood after exposure to the partial vacuum is transferred to a simple form of differential blood gas manometer in which there are no glass stopcocks, pressure adjustment being made by the use of the pressure adjuster described elsewhere.[3] Even with these simplifications, the technic is by no means easy, but the great importance of having the student clearly understand the principles of the method used for securing the data necessary to plot the dissociation curve amply repays the time he devotes to the experiment. Indeed without actually doing this experiment, it is the opinion of the writers that the average student rarely acquires any clear conception of the respiratory function of the blood. After he has conscientiously performed the experiment, on the other hand, the whole problem becomes clearer, even although results that are

absolutely correct may not have been secured. A laboratory session of at least three continuous hours is essential, and the students can most profitably work in groups of three each.

## DESCRIPTION OF THE APPARATUS

*The Tonometer.*—This consists of a wide glass tube (the tonometer *T*, Fig. 1) of fairly stout glass, tapering down to narrow tubes at both ends. The capacity should be at least 200 c.c.* The narrow tubes are connected with thick-walled (pressure) rubber tubing which should be wired on to the glass tubes. The rubber tubes are closed by screw clips (1 and 2). File marks are made at

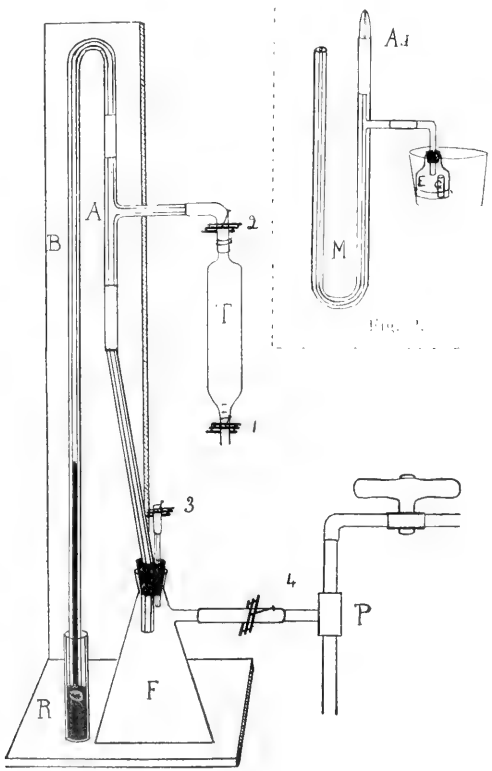


Fig. 1.

one of the tapering ends of the tonometer, the distances between them corresponding approximately to one cubic centimeter.

*The Barometer.*—This consists of a vertical, thick-walled glass tube about 1.25 meters long and of about 3 mm. bore bent on itself near one end, and with the other end dipping into mercury contained in a wider flat-bottomed (specimen) tube (the mercury reservoir) closed by a perforated cork. The barometer tube and reservoir are firmly mounted on a stand furnished with a millimeter scale which is attached to the stand in such a way that it can be adjusted to bring its zero to the surface of mercury in the reservoir, as this varies at different pressures. The free end of the barometer tube is connected by rubber pres-

*It would be preferable to use a tonometer two times as large since this would diminish errors due to the addition of the gas given off from the blood.

sure tubing to a glass T-piece (*A*), one limb of which is similarly connected to a stout-walled (filtration) flask (*F*) joined to a good water pump (*P*). A capillary tube closed by a piece of rubber tubing and a screw clip (*3*) also passes through the stopper of the flask.

*The Differential Manometer and Gas Bottle.*—This apparatus requires a piece of narrow bored glass tubing (bore 1 mm.) bent into a U-shape with one limb about 200 mm. long and the other about 150 mm. (*M* in illustration). To the shorter limb is connected a T-piece (of narrow bore tubing) which should be fused to it, although rubber pressure tubing is quite satisfactory. One limb of the T-piece is connected with red rubber tubing of moderately thick wall, closed by a pressure adjuster (Ad) as described elsewhere.* By pinching the rubber tubing lying over the lateral hole of the adjuster, the manometer is brought into communication with the outside so that the air in the bottle and manometer are brought to atmospheric pressure. The other tube of the T-piece is connected by pressure rubber tubing with a glass tube which passes through a rubber stopper that accurately fits a small wide-mouthed bottle *E* of about 15 c.c. capacity. Finally a small flat-bottomed test tube 15 mm. high and 6-7 mm. diameter is required, *I*. Instead of attaching the T-piece to the short limb of the manometer, it may be inserted in the rubber stopper, one limb being connected with the manometer—which in this case simply consists of a U-tube—and the other with the pressure adjuster. The fluid used in the manometer is clove oil.

## TECHNIC OF ANALYSIS

The tonometer is rinsed out with physiologic saline and is connected with the side tube of the barometer T-piece *A*. The pump *P* is turned on with screw clips *1* and *3* closed, but screw clip *2* open and the pressure lowered until the mercury stands at a constant level in the barometer. Screw clip *4* is closed and the mercury observed to see whether there is any leak. Provided there is none, clip *3* is cautiously opened and the mercury allowed to fall almost to the level in the reservoir (*R*); clip *2* is tightened, the tonometer, *T*, removed, and the pump turned off. Defibrinated or oxalate blood (whipped ox blood is most suitable for large classes, but in any case blood from an etherized animal must not be used) is now sucked into the tonometer, by placing some of the blood in a small evaporating dish and cautiously loosening clip *1* with the rubber tube dipping into the blood; *3* to *4* c.c. of blood should be allowed to enter the tonometer. This is then reattached to the T-piece *A* of the barometer and with clips *2* and *4* open (but *1* and *3* closed) the pump is turned on and the mercury allowed to rise as far as it will go, when clip *4* is closed and the pump turned off. Clip *3* is now cautiously opened until there is a partial pressure of about 20 mm. Hg oxygen in the tonometer.†

*This Journal, iv, 69.

†This is computed as follows: After suitable adjustment the standard barometer in the room is read and from the reading is subtracted the tension of aqueous vapor at the temperature of the room (for table see page 420 of this Journal, Vol. III). The difference gives the pressure in mm. Hg of an atmosphere of dry air. Since air contains 20.96 per cent oxygen, the partial pressure of this gas in the tonometer must be equal to $\frac{20.96}{100}$ of the difference between the height to which the mercury is raised in *B* and the corrected barometer reading. Thus, suppose the room barometer is 753.4 mm. and aq. tension 17.4 mm. the corrected barometer reading is 753.4-17—4 = 736 mm. Then $\frac{20.96}{100} \times 736 = 154.2$ mm. $O_2$.

$$20 \text{ mm. } O_2 = \frac{20 \times 736}{154.2} = 95.45 \text{ mm.}$$

That is the mercury in the barometer must be raised to 736—95.45 640.55 mm.—above the level in the reservoir (*R*).

When the mercury has reached this level, or one near it, clip *3* is closed and the height at which the mercury stands very accurately noted. Clip *2* is then closed, after which the mercury is allowed to fall to zero by opening *3*. The tonometer is now removed and rotated so that the blood becomes spread out as a thin film on the walls, after which it is placed in a water-bath kept at about 40° C. in which it is constantly rotated for about 15 minutes.

On removal from the bath the pressure in the tonometer must again be measured. For this purpose the tonometer is reattached to *A* and the pump is turned on (with *3* closed) until the mercury has risen to the level at which it previously stood. Clip *4* is closed and *2* opened. If there has been no leak, and time has been allowed for the tonometer to cool down, there will be practically no difference between the two readings. If a difference of more than 5 mm. is observed it must be noted and the pressure prevailing in the tonometer taken as the average between the two readings.

Meanwhile 3 c.c. of freshly prepared weak ammonia water containing a trace of saponin (0.5 c.c. aq. ammonia in 500 c.c. water) has been placed in the blood gas bottle *B*. A pointed glass tube about 30 mm. long is now attached to the rubber tubing of the tonometer and this is removed from the barometer and held in vertical position above the bottle. The screw clip *2* is opened so that the air enters the tonometer, the clip *1*, is then cautiously opened to let a drop or two of blood flow out from the tip of the glass tube,* and after closing it again the end of the tube is wiped free of blood and placed in the bottle so that it dips under the ammonia solution. Clip *1* is now cautiously opened and about 1 c.c. of blood allowed to flow under the ammonia water. If this is done carefully the blood does not mix with the ammonia water which floats on the top of it as a layer and so prevents any diffusion of oxygen between the blood and the air. The bottle is firmly closed by its stopper, the pressure adjuster being meanwhile held open so that the level of the clove oil in the manometer is not disturbed. The bottle must now be submerged in a water-bath containing water at about room temperature, in which it is left until, with the adjuster closed, no further contraction of volume, due to cooling, is observed to occur.

The bottle is now removed from the bath and vigorously shaken so that the blood becomes laked and absorbs $O_2$ from the atmosphere of the bottle. After replacing the bottle in the bath and allowing time for cooling the difference between the levels of clove oil in the two limbs of the manometer is noted. With the adjuster open to the outside the stopper is removed from the bottle and about 0.25 c.c. of a freshly prepared saturated solution of potassium ferricyanide is placed in the small flat-bottomed test tube which is then lowered by means of a forceps into the fluid in the bottle, without allowing any of the ferricyanide to mix with the laked blood. After reinserting the stopper and cooling, the bottle is again removed from the bath and shaken so that the ferricyanide by mixing with the laked blood drives off the loosely combined oxygen and raises the pressure, which is measured by the manometer.

The relative amounts of reduced and oxy-hemoglobin present in the blood are proportional to the first and second readings of the manometer; when all is reduced hemoglobin the diminished pressure (shrinkage) recorded in the first

*Enough blood should be run out to bring the meniscus of blood in the tonometer to the upper file mark.

shaking of the bottle is practically the same as the increased pressure recorded in the second. They will not be exactly the same, since the volumes of bottle and tubing in the two cases are not the same, but the error thus incurred is inconsequential for most purposes (cf. Boyle's law).

The calculation of the percentage saturation of hemoglobin with oxygen is made by subtracting the first reading from the second, dividing by the second reading and multiplying by 100. Suppose in the observation made at 20 mm. partial pressure of $O_2$ the first reading is 24 mm. and the second, 108, then

$$\frac{108-24}{108} \times 100 = 77.7\% \text{ HbO and } 22.3\% \text{ Hb.}$$

The result must now be plotted on coordinate paper with the percentages of HbO along the ordinates and the partial pressures of oxygen on the abscissæ.

The observation is repeated at different pressures and by joining the points, the dissociation curve for blood is obtained. Care must be taken to see that the bottle is sufficiently shaken so that the partly reduced blood absorbs all the oxygen and gives it up again with ferricyanide. It is particularly in the latter operation that care must be taken.

*The influence of carbon dioxide in lowering the dissociation curve* can be readily shown by the method. The procedure is as follows: After the pressure has been reduced to the desired degree in the tonometer, the latter is placed in a horizontal position so that the blood lies along the walls, free of the ends. A $CO_2$ generating apparatus (Kipp's) or a bottle containing this gas is then connected by suitable tubing with the free end of the tonometer, care being taken before making the connection, to fill the tubing with $CO_2$. To accomplish this a slow stream of the gas is maintained and the air in the tubing beyond the screw clip (*1*) is squeezed out before connecting with the $CO_2$ generator. The most suitable partial pressure of $CO_2$ to work with is 40 mm. which is secured by cautiously opening screw clip *1* until with clip *2* open, but *3* and *4* closed, the mercury descends through 40 mm. in the barometer. Clips *1* and *2* are then tightly screwed down, and the tonometer removed, the further procedure being exactly as described above.

The effect of the 40 mm. of $CO_2$ will be found in the above example where a partial pressure of 20 mm. $O_2$ was used to reduce the percentage of HbO from 77 to about 35.

### THE $CO_2$-COMBINING POWER OF THE ALKALINE RESERVE OF THE BLOOD

After completing the estimations necessary for finding the percentage of oxyhemoglobin, in the experiments in which $CO_2$ is present in the tonometer, it is of interest to determine the amount of this gas with which the blood has combined. This will represent its ability to act as a buffer towards foreign acids. To perform the estimation it is necessary, however, to measure accurately the amount of blood which is removed from the tonometer to the blood gas bottle. This can readily be done by attaching a 1 c.c. pipette to the tubing of the tonometer (beyond clip *1*), a few drops of blood being allowed to escape from the pipette before delivering under the ammonia solution in the bottle, and precautions being taken not to take any of the upper layers of blood that had been exposed to full

atmospheric pressure when the tonometer was opened. This is done by removing the pipette from the tonometer before all the blood has run out.

To dislodge the $CO_2$ from the blood, the stopper is removed with the usual precautions and about 0.25 c.c. of a saturated solution of tartaric acid placed in the small test tube. After closing and allowing for temperature changes, the acid is shaken with the mixture of blood and ferricyanide, and the $CO_2$ thereby evolved measured by multiplying the displacement of the fluid in the manometer by a figure (the constant of the apparatus) obtained by a preliminary experiment in which a known amount of a standard carbonate solution is similarly treated.

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# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES


No. 25: OBSERVATIONS ON DECEREBRATE CATS, BY LOIS FRASER, R. S. LANG and J. J. R. MACLEOD

(REPRINTED FROM THE AMERICAN JOURNAL OF PHYSIOLOGY, VOL. 49)

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*Observations on decerebrate cats.* Lois Fraser, R. S. Lang and J. J. R. Macleod.

It has been shown by one of us that the respiratory volume remains fairly uniform for three or four hours in most decerebrate cats after the effects of the initial etherization have passed off. Hyperpnoea usually develops later and this is accompanied by a depression in the alveolar $CO_2$ percentage and a lowering of the arterial blood carbonate. In the present investigation it was intended to study the effect produced by causing the animal to breathe through valves into a closed system of wide-bore tubing provided with soda-lime absorption bottles and a Gad-Krogh spirometer. The object was to find the exact degree of oxygen deficiency at which increased pulmonary ventilation would supervene, and to seek for evidence as to whether this hyperpnoea is associated with changes in the alveolar air (R.Q.) and blood (arterial-blood $CO_2$ + PH) that could be attributed to the appearance in the organism of unoxidized acid.

Although a sufficient number of data has not as yet been collected to answer these questions, the interesting observation has been made that a decided hyperpnoea develops a few minutes after connecting the animal with the respiration tube and spirometer. The evidence of this hyperpnoea is obtained partly by observing the tracing produced by the spirometer, or by a tambour connected with the tracheal tube before the valves, and partly by analysis of the alveolar air. The results with the latter have invariably shown a decrease in the percentage of $CO_2$ and a decided rise in the respiratory quotient, while the oxygen in the inspired air is still well above 15 per cent.

Similar results were obtained in two experiments in which an excess of oxygen was added to the system before causing the animal to respire into it. There can be no doubt that the slight resistance offered to expiration has served to increase the excitability of the respiratory center. This may be due to afferent stimuli set up either by the more distended condition of the alveoli (acting on the center through the vagi) or by the distended condition of the thoracic walls (acting through the muscle nerves). The following figures will serve to illustrate the results.

| Number of Experiment | Respiratory Volume in Cubic Centimeters per Minute | | Respiratory Quotient | | $O_2$ Percentage in Alveolar Air During Observations |
|---|---|---|---|---|---|
| | Before | During | Before | During | |
| XXIV | 1080 | 1845 | 0.82 | 1.63 | 14.4 |
| XXVIII | 600 | 1200 | 0.69 | 1.58 | 15.34 |
| XXVI | 905 | 2000 | 0.85 | 1.02 | 15.25 |
| XXIX | 1120 | 2250 | 0.85 | 1.27 | 17.7 |

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PHYSIOLOGICAL SERIES

No. 26: DEATH PRODUCED BY TYING THE ADRENAL VEINS, BY F. A. HARTMAN and W. E. BLATZ

(REPRINTED FROM ENDOCRINOLOGY, VOL. 3)


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# DEATH PRODUCED BY TYING THE ADRENAL VEINS

F. A. Hartman and W. E. Blatz

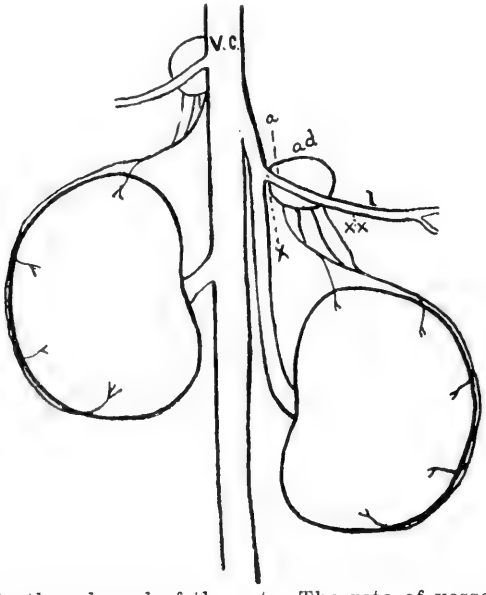
(From the Department of Physiology of the University of Toronto)

Since the discovery that Addison's Disease is due to disturbance of the adrenal apparatus, many attempts have been made to produce the disease artificially. Adrenal insufficiency is said to be the main cause, and hence epinephrectomy was thought to be the best method of producing the peculiar syndrome of the disease. This method is, however, too drastic, the animal succumbing more or less rapidly to the operation. Some method of reducing the adrenal function without completely destroying it, appeared to be required.

Certain infectious diseases impair the function of the adrenals. Diphtheria (1) produces vacuolization and hemorrhage in the adrenals, and diphtheria toxin (2) is said to lessen the pupil-dilating substance in the adrenal venous blood. In view of this we attempted to destroy a portion of each gland by the injection of sublethal doses of diphtheria toxin into the exposed gland. Evidently the toxin was either neutralized, or else washed away by the blood stream before it could cause much destruction of the adrenal tissue, because no symptoms could be noticed, following such injection. Although we experimented with only one cat and one guinea pig, the method was considered unsatisfactory.

The blood flow through the adrenals is relatively very large; therefore by hindering the blood supply we should be able to produce degenerative changes rather easily. If the blood flow could be almost stopped, the changes in the gland might be slow enough to produce merely a condition of hyposecretion, such as we desired. The arteries break up into such fine branches before entering the capsule, that checking the inflow would be too difficult. On the other hand, the outflow is mainly through a large vein emptying into either the vena cava, or else the renal vein, so that by ligation the flow could easily be stopped. However, a small amount of blood escapes

through a rete of vessels connecting the adrenal with the kidney (3) so that occlusion of the adrenal vein does not produce complete stasis of blood in the gland. (See accompanying figure.)




Veins to the adrenal of the cat. The rete of vessels connecting the adrenal with the kidney, after the diagram of Cow.
ad., adrenal.
a., common lumbo-adrenal vein.
l., lumbar vein joining the adrenal vein.
v. c., vena cava.
x., position of ligature.
xx., ligature here in some experiments, in addition to position x.

This research is a preliminary study of the effects produced by disturbing the blood supply to the adrenals.

## Methods

A lumbar vein from the dorsal musculature joins the adrenal vein as the latter leaves the gland (Fig.) so that their blood is carried by a common trunk into the vena cava. In some cases one adrenal was excised. In all of the experiments the common lumbo-adrenal vein was completely tied off (see X in Fig.) central to the adrenal or adrenals left in the body.

Because of the possibility of a back flow through the lumbar vein, this was also tied in a few animals (XX, Fig.). All

animals were anesthetized with ether and the operations conducted aseptically. Cats were used in a majority of cases, but dogs and rabbits occasionally.

After the operation the animals were studied at first daily, and then at longer intervals to see whether characteristic symptoms developed. They were examined for changes in rectal temperature, weight, heart rate and muscular weakness. In some cases the animal was killed, in others it was allowed to die following the natural course of events. The adrenals were then fixed and studied.

The glands were placed for twenty-four hours in a mixture of 90 parts of 3.5 per cent $K_2Cr_2O_7$ with 10 parts of 40 per cent formaldehyde (4). They were then washed in running water for an equal time, after which they were immersed in a solution of Gum Arabic for a few hours before sectioning.

Sections 5 micra to 30 micra in thickness were cut with a freezing microtome. After washing in tap water to remove the gum, the sections were stained 5 to 10 minutes with Delafield's haemotoxylin, subsequent treatment with water removing the excess stain. They were finally left in a saturated 70 per cent alcoholic solution of Sudan III over night. After again washing with water they were mounted for study in Farrant's medium. The lipoids were stained red by Sudan III and chromaffin cells yellowish if they contained adrenalin.

## Results

**Experiment 1.** The adrenal veins to both glands in a cat were tied off by ligating the vein coming from the lumbar muscles and the common vein as it entered the vena cava. The only possible outlet from the adrenals was the kidney rete. Forty-eight days later the animal died without developing any noticeable symptoms except muscular weakness just before death. Microscopic examination of the glands showed a slight vacuolization in the zona fasciculata and the presence of adrenalin in the medulla. Lipoids were plentiful in the cortex.

**Experiment 2.** The right adrenal was excised and both the lumbar vein and the common lumbo-adrenal vein tied off on the other side. The cat died two days later with no observed external change. The adrenal which had remained in the animal was much congested with blood. The superficial veins of

the kidney on the same side were also congested. The two glands were compared microscopically. The appearance of the left adrenal was very striking. Adrenalin instead of being limited to the medulla was disseminated throughout the gland, being especially concentrated in the cells of the zona glomerulosa. Adrenalin in the zona fasciculata and zona reticularis was found between the cell columns, but was absent from the cells. The total quantity of adrenalin was much greater in the left adrenal than in the right, as indicated by the staining. There was considerable vacuolization in the zona fasciculata.

**Experiment 3.** The lumbar and lumbo-adrenal veins were tied on the left side, the right adrenal being left intact. The animal in this case was a dog. Eighteen days later the animal was killed, and the two adrenals fixed. No great difference was observed between the two glands. There was less lipoid in the ligated gland, there being great numbers of large lipoid bodies in the cortex of the normal gland, while these were largely absent from the ligated gland.

**Experiment 4.** The lumbo-adrenal vein where it entered the vena cava was tied off on both sides, but the veins to the lumbar muscles were not touched. This animal, a rabbit, gained in weight during the next thirty-two days. It died seventy-five days after the operation. Upon microscopic examination the presence of adrenalin was not found. Lipoids were very scant in amount.

**Experiment 5.** After removal of the right adrenal the lumbo-adrenal vein from the left gland was tied off, the vein from the lumbar muscles being left intact. The animal (cat) was operated upon at 4 p. m.

At nine o'clock the next morning the pupils were still greatly dilated, and constricted much less than did those of a normal cat when exposed to a bright light. The most striking thing, however, was the marked pilomotor effect. The hair was erect all along the dorsal surface, and to a certain extent, on the tail. The heart was beating at the rate of 140 per minute, whereas before the operation the rate was 250. The rectal temperature was 35.7° C.

Forty-one hours after the operation, the pupils had become nearly normal, while the pilomotor effect still persisted, but

was not quite so marked. The heart rate was 184 beats per minute. The rectal temperature was 37.2° C.

In four days the cat appeared normal except for a slight pilomotor effect. Later certain changes were apparent. The hair on the face was falling out. The hair on the ears had become quite scanty. There was a red coloration on the inner side of both forelegs. The cat began to cry incessantly and lost weight. Eruptions appeared on the skin of the face (46 days after the operation).

Sixty-eight days after the operation, the weight had been reduced from 2.500 kgm. to 2.060 kgm., in spite of its eating well. The rectal temperature was 39.1° C. The hair was very scraggy and unkempt. The cat was irritable, but weaker than normal. It was not so active, largely on account of muscular weakness, because it still appeared restless. The heart rate was normal (249). At this time there was considerable difficulty in micturating. It took several minutes to expel the urine.

Ninety-five days after the operation, the heart was still normal in rate, the temperature still high (39.0° C.) and the weight partly regained (2.350 kgm.). It appeared a little stronger, but still walked with a stiff-legged gait. The fur was still in a poor condition, the longer hairs, such as the vibrissae, being broken and scraggy.

The cat died 128 days after the operation. It had been gradually losing weight so that it was reduced to two-thirds the original, viz., to 1.750 kgm. The day before death it appeared very much as it had for weeks. (Death was hastened the last two or three days by a marked fall in the room temperature.)

Upon post mortem everything was found well healed. The lumbar vein to the left adrenal was much enlarged. The adrenal weighed 0.426 gm. Lipoids were fairly plentiful in the gland, but did not seem to be entirely confined to the cortex. Some adrenalin was present, as shown by the yellow stain.

The kidney on the same side had been fixed and stained in the same manner as the adrenal. Many of the blood vessels in the outer zone were stained yellow. Scattered here and there tubules and glomeruli were found similarly stained. It appeared that adrenalin brought to the kidney directly from

the adrenal was being excreted in the kidney. However, this observation needs further confirmation.

**Experiment 6.** A second cat was operated upon in the same way as in the preceding experiment. Within two hours after the operation the pupil had become normal. Eight hours later there was a decided pilomotor reaction along the back. In 13 hours the pilomotor effect had become slight. No other changes appeared. The animal died 18 days later. It had been eating well up to that time.

There were only slight traces of lipoid in the cortex of the adrenal which had been left in the animal. There were also traces of lipoid in the medulla. Adrenalin if present at all was extremely scant.

**Experiment 7.** The adrenals of a third cat were treated as in the two foregoing cases. Three hours after, there was a marked pilomotor effect, but no dilatation of the pupil.

The pilomotor effect persisted for 38 days. At that time the animal appeared weaker. The temperature and heart rate remained about normal. The animal died 59 days after the operation. It had fallen off slightly in weight, from 1.34 kgm. to 1.22 kgm.

Histologically, the adrenal which had remained in the animal was almost devoid of lipoids, the small amount present being in the cortex. No trace of adrenalin was found.

**Experiment 8.** A dog was operated upon as in the preceding experiments. It recovered without showing any decided symptoms. The rectal temperature 10 days after the operation was 39.2° C. The animal was killed 83 days after the operation. Its weight had fallen from 5.1 kgm. to 4.6 kgm. Histologically, the adrenal gland which had remained in the animal differed very little from normal.

**Experiment 9.** In order to determine how much pressure might be produced in the lumbar vein when the lumbo-adrenal vein was tied, we fastened a cannula into the former vein immediately after the latter had been tied. A large dog (18 kgm.) was used while under ether anesthesia. The cannula was connected with a long vertical glass tube containing a little half-saturated $Na_2SO_4$ solution.

The pressure in the tube gradually rose as follows:

| Time in minutes | Increase mm. ½ sat. $Na_2SO_4$ Sol. | Total pressure mm. ½ sat. $Na_2SO_4$ Sol. |
|---|---|---|
| 1 | 155 | 155 |
| 2 | 109 | 264 |
| 3 | 93 | 357 |
| 4 | 81 | 438 |
| 5 | 71 | 509 |
| 6 | 60 | 569 |
| 7 | 47 | 616 |
| 8 | 38 | 654 |
| 9 | 30 | 684 |
| 10 | 26 | 710 |
| 11 | 19 | 729 |
| 12 | 19 | 748 |
| 13 | 12 | 760 |
| 14 | 9 | 769 |
| 15 | 6 | 775 |

The final pressure attained in the glass tube was 775 mm. or 73.25 mm. in terms of Hg. Arterial pressure at the same time was 77 mm. of Hg.

## Discussion

The method which we have used does not produce death nearly so quickly as does the removal of both adrenals. In that case death occurs in a few hours to a few days (5). Six of the seven animals, in which the veins from the adrenals had been tied, died in 2, 18, 48, 59, 75 and 128 days respectively. Experiment No. 3 should not be included because one gland was left intact in the animal. Experiment No. 8, on a dog, was the exception, the animal being killed after 83 days. At the time of death, however, it was losing weight.

Three of the animals which died of their own accord, showed symptoms of weakness some time before death; in the other three, no changes were observed. In regard to the histological changes, three of the animals showed no presence of adrenalin, and only a trace of lipoid in the adrenal after death, while three others showed both adrenalin and lipoid in larger amount. Of course, the absence of adrenalin in a gland which

is not fixed immediately at death indicates little, for adrenalin disappears very soon. But in any case, judging from the lipoids, with the exception of experiment No. 5, the adrenal function was much below normal.

Cat No. 5 was very exceptional and may have been a case of hyperactivity of the adrenal. Many of the symptoms tend to indicate that. It is quite possible that in this instance the increased pressure produced by tying the common lumbo-adrenal vein stimulated the cells of the adrenal to greater activity. The increased pressure may also have produced a back-flow of blood through the lumbar vein and thus through anastomoses into the general circulation. In fact, this was possible in all the animals where the lumbar vein was not tied, because the pressure attained is nearly as great as arterial pressure (experiment No. 9).

**Summary**

1. After having the veins to the adrenal glands tied an animal lives much longer than after double epinephrectomy, but eventually dies.

2. There is evidence that the adrenals function for a considerable time after such an operation, the secretion escaping through the rete of vessels leading to the kidney, and possibly by back flow through the lumbar vein, when that is left open.

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UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES


No. 27: ACTION OF ADRENALIN ON THE SPLEEN, BY F. A. HARTMAN and ROSS S. LANG

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# University of Toronto Studies

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# ACTION OF ADRENALIN ON THE SPLEEN

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It is generally agreed by recent investigators (1) that adrenalin causes dilatation of the blood vessels in certain parts of the organism. It has been found from experiments carried out in this laboratory that this dilatation is caused, at least in part, by the action upon sympathetic and dorsal root ganglia (2). It may also be caused as shown by Gruber (3) and confirmed by us (4) that the dilatation may result from the stimulation of some peripheral tissue, perhaps myoneural junctions of dilator fibres. It is not possible to say which is more important in producing dilatation normally, the "gangliar" mechanism or the myoneural junction. In some animals the same amount of dilatation has been obtained by the action of adrenalin upon the gangliar portion of the mechanism alone (limb perfused, adrenalin injected into the jugular vein) as occurred from the injection of the same quantity when the circulation was intact (4).

Cats and dogs have been used principally for adrenalin experiments, but work recently published from this laboratory (5) has shown this reaction to be common to Marsupials, Ungulates, and Primates, Rodents being an exception. Therefore physiologists can no longer dismiss adrenalin vasodilatation as an interesting exception.

A careful study has been made of the "gangliar-terminal" action in the hind limb (4), but in many other organs this has not been done. It is the purpose of this and succeeding researches to make a further study of this question in various organs. The present paper is confined to the spleen.

## METHODS

Volume changes in the spleen were recorded by enclosing the organ in a gutta percha oncometer connected to a bellows of the Brodie type. The flexible part of the latter was made of rubber cut from a condom. This was fastened to the edge of the bellows base and top with thin glue except at the back where the hinge is located, and where the overlap occurs rubber cement must be used, because the glue when dry stiffens the rubber. Formerly rubber cement was used throughout, but the curling which it causes renders the bellows very difficult to make. The lever for the writing point was attached at right angles to the top of the bellows.

The nerves and blood vessels were carefully freed from fat and connective tissue and then grouped so as to form a double stalk. In many cases it was possible to do this without tying any blood vessels. The spleen was placed in a double-necked oncometer (1e) which was covered with a glass plate connected to the transmission tube.

For perfusion we used one of the large arteries which supplied about one-half of the spleen, the remaining arteries being tied off. Warmed oxygenated Ringer's solution was perfused under a constant pressure produced by compressed air. The temperature and pressure of the perfusion fluid were registered at the entrance to the cannula.

Ether was used as the anaesthetic. Adrenalin solutions were made by diluting Parke, Davis and Company's adrenalin chloride solution with distilled water.

## RESULTS

In an earlier research (1e) we made a careful study of the normal spleen in its reaction to adrenalin injected intravenously; seven dogs gave nothing but constriction, while three others responded by dilatation or dilatation and constriction. There seemed to be some question as to the occurrence of active dilatation in the spleen, because in two of the animals, the dilatation preceded the constriction and therefore might be a passive

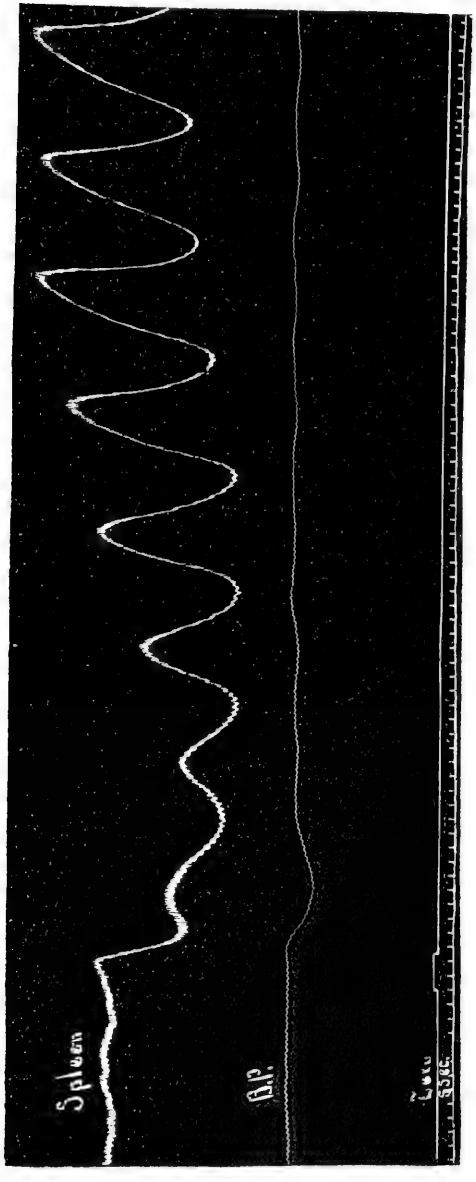


FIG. 1. WAVES PRODUCED IN A PRACTICALLY QUIESCENT SPLEEN BY THE INJECTION OF A DEPRESSOR DOSE OF ADRENALIN, 0.2 CC., 1:100,000. CAT 2.5 KGM.

effect due to constriction elsewhere. Dilatation followed constriction in only one spleen and that was after a large dose of adrenalin.

We have studied four more normal spleens, two of the dog, two of the cat; all but one cat gave dilatation with some dose of adrenalin. In this animal (2.5 kgm.) no dilatation could be secured from a range of doses starting with 0.1 cc., 1:100,000 adrenalin and running as high as 0.5 cc., 1:10,000, however after many of the injections the amplitude of the splenic waves

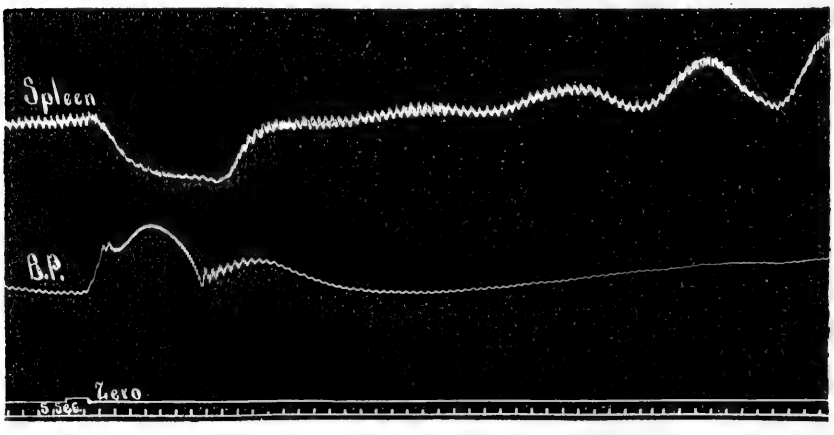


FIG. 2. WAVES PRODUCED IN A QUIESCENT SPLEEN BY THE INJECTION OF A PRESSOR DOSE OF ADRENALIN, 0.5 CC., 1:10,000. CAT 2.5 KGM.

was increased, although the initial effect might be a partial inhibition of the waves. Again the splenic waves might be practically absent until adrenalin was injected, after which they became very marked (fig. 1). Even pressor doses produced a similar effect (fig. 2).

In a second cat (3.7 kgm.) slight dilatation always preceded the constriction which in many cases was followed by waves. Small doses such as 0.1 cc., to 0.5 cc., 1:10,000,000 caused dilatation only.

Although dilatation usually preceded constriction in the two dogs, it occasionally followed the constriction in one animal.

It seemed possible that dilatation of the spleen as in the intestine might be caused by stimulation of the ganglia supplying it. In order to find out whether structures not located in the spleen, could be the cause of the dilatation, we tied all of the blood vessels, and then perfused a portion of it through one of the largest arteries, the outflow being from a vein which had been cut open. Great care was taken to preserve the nerve supply. In this way the effects of adrenalin could be observe either solely upon structures in the spleen by injection into the perfusion fluid or upon structures located outside of the spleen by injection into the general circulation.

We perfused three spleens. The first belonged to a dog weighing 22 kgm. and gave the following responses:

| DOSE | PLACE OF INJECTION | RESPONSE IN BLOOD PRESSURE IN MM. OF MERCURY | RESPONSE OF THE SPLEEN |
|---|---|---|---|
| 1.0 cc. 1: 100,000 | Jugular vein | 180–186–158 | Dilatation |
| 3.0 cc. 1: 100,000 | Jugular vein | 177–190–154 | Dilatation |
| 5.0 cc. 1: 100,000 | Jugular vein | 188–194–164 | Marked dilatation |
| 1.0 cc. 1: 10,000 | Jugular vein | 188–210–168 | Marked dilatation |
| 0.2 cc. 1: 1,000,000 | Through perfusion fluid | None | Constriction, very marked |
| 0.1 cc. 1: 1,000,000 | Through perfusion fluid | None | Constriction, marked |
| 0.1 cc. 1: 10,000,000 | Through perfusion fluid | None | No effect |

The perfused spleen of this animal dilated with every dose of adrenalin injected into the jugular vein (fig. 3), but constricted with each injection into the perfusion fluid (fig. 4). The latter was injected into the fluid just before it entered the cannula. The pressure for perfusion was 45 mm. of mercury while the temperature was 33.4°C.

The second spleen did not seem to be responding very well and no effect could be obtained except a slight constriction when adrenalin was introduced into the general circulation.

The third perfused spleen, belonging to a dog weighing 10 kgm., dilated considerably with the doses of adrenalin injected

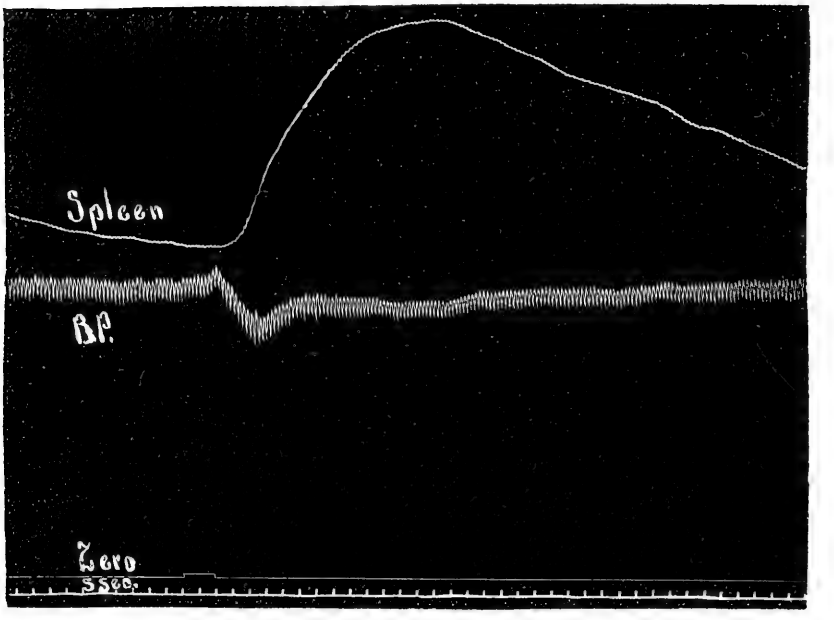


FIG. 3. DILATATION OF A PERFUSED SPLEEN FROM THE INJECTION OF 5.0 CC. 1: 100,000 ADRENALIN INTO THE JUGULAR VEIN. DOG 22 KGM.

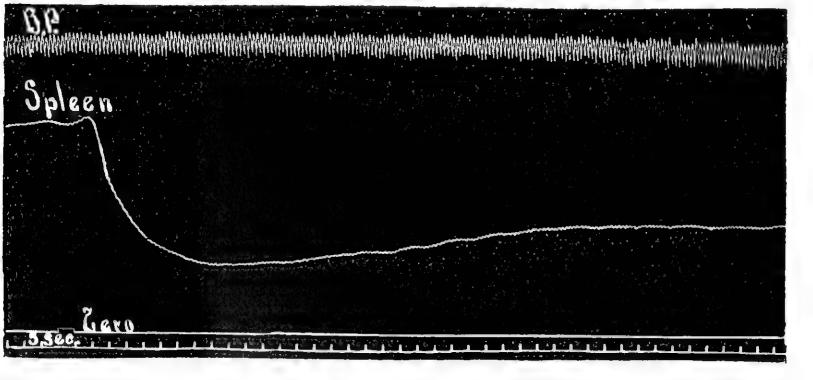


FIG. 4. CONSTRICTION OF A PERFUSED SPLEEN FROM THE INJECTION OF 0.1 CC., 1: 1,000,000 ADRENALIN INTO THE PERFUSION FLUID. DOG 22 KGM.

into the jugular vein, 0.5 cc., 1: 100,000 to 0.5 cc., 1: 10,000. These doses were depressor in their effect upon the blood pressure. When adrenalin was injected into the perfusion fluid, constriction was followed by dilatation (fig. 5). This was true even with a relatively large dose, 0.2 cc. 1: 100,000. The dilatation, however, was not as marked as that produced from the mechanisms outside of the spleen.

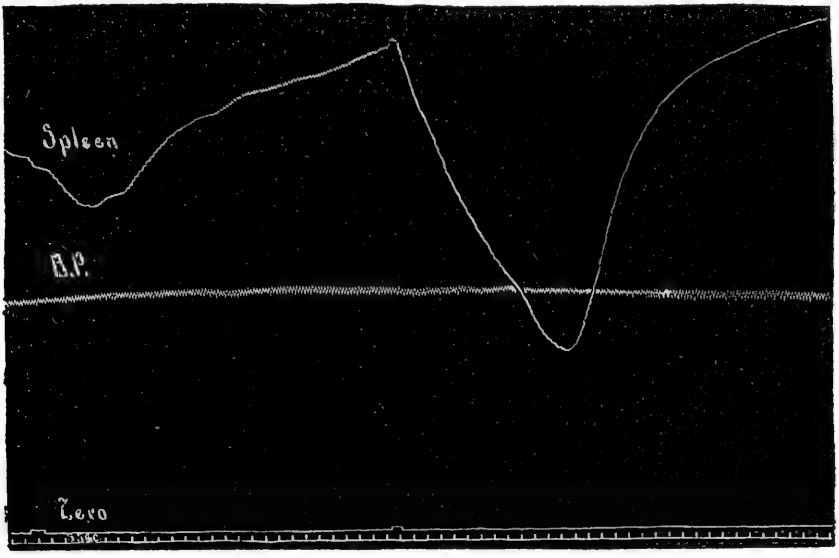


FIG. 5. CONSTRICTION FOLLOWED BY DILATATION, PRODUCED BY THE INJECTION OF ADRENALIN INTO THE PERFUSION FLUID ENTERING A PERFUSED SPLEEN.

First injection 0.2 cc., 1: 1,000,000; second injection, 0.2 cc., 1: 100,000. Dog 10 kgm.

In order to determine whether the semilunar ganglion contained mechanisms which might cause dilatation of the spleen through the action of adrenalin, direct application of adrenalin to this ganglion was tried while the spleen was in an oncometer with its circulation intact. If no changes in blood pressure occurred during the experiment we were justified in assuming that adrenalin was not passing into the blood stream and there-

fore could produce its effect only by gangliar action. Absorption was facilitated by slitting the surface of the ganglion.

The spleen of a cat was studied by this method. Solutions of 1:100,000 were twice applied without changing the blood pressure, but in each instance causing dilatation of the spleen. A third application of a stronger solution caused a very marked dilatation (fig. 6).

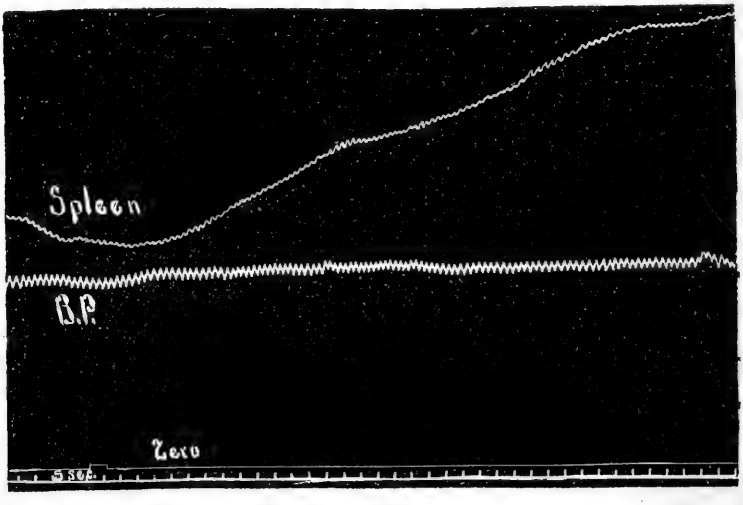


FIG. 6. DILATATION OF THE SPLEEN CAUSED BY THE DIRECT APPLICATION OF 1:10,000 ADRENALIN TO THE SEMILUNAR GANGLION. CAT.

This experiment therefore demonstrates that the semilunar ganglion is one location of the adrenalin dilator mechanism of the spleen.

We also studied the effect of adrenalin upon the spleen through action upon the ganglia of the dorsal nerve roots. These ganglia were exposed and painted with solutions of adrenalin after cutting the connections with the spinal cord. In some instances the ganglia were split open to facilitate absorption. Blood pressure records were taken at the same time. Care must be taken not to stimulate the ganglia mechanically, for sometimes that will cause splenic volume changes. The twelfth and thirteenth thoracic ganglia on the left side were almost always used.

Five cats were studied. All showed some response to adrenalin applied to the above ganglia, although in some instances only one response could be obtained from a single ganglion, a new ganglion being required in that case to secure a repetition of the response. The volume changes are usually slow in occurring and likewise slow in disappearing unless the ganglion is washed to remove the adrenalin.

One animal responded by dilatation only with concentrations of 1: 10,000 and 1: 1000; weaker solutions were not tried.

Three of them gave dilatation sometimes and constriction at other times, there being no regularity in the occurrence of either.

The fifth cat was interesting in that adrenalin applied to the ganglia in question, caused waves in the spleen if it were quiescent, or increased the amplitude of the waves if they were already present.

We would conclude from our observations that both constrictor and dilator mechanisms for the spleen are present in the dorsal root ganglia. We cannot say which predominates.

## DISCUSSION

Oliver and Schäfer (6) were the first to study the action of adrenalin (adrenal extract) upon the spleen. In no cases did they obtain a dilatation except "a very slight preliminary expansion," probably caused by the increased heart's action. A later paper by Schäfer and Moore (7) added to this observation that the after effect of the injection was to increase the extent of the normal rhythmic movements. When injected into a perfused spleen a strong contraction was obtained.

Bardier and Fränkel (8) obtained dilatation from macerated adrenals. Others (9) speak only of contraction of the spleen from adrenalin.

Recently Hoskins and Gunning (1c) and Hartman and McPhedran (1e) have observed mainly constriction from adrenalin in the spleen. The former speak of a brief dilatation followed by contraction. They occasionally obtained an active dilatation following the constriction.

Our experiments have proven that dilatation from adrenalin can be obtained by action upon structures in the semilunar ganglion, and the dorsal root ganglia as well as by action upon structures in the spleen. In this respect the adrenalin dilator mechanism of the spleen is similar to that of skeletal muscle (as shown by the hind limb, 4).

There can now be no doubt that adrenalin produces active dilatation of the spleen. Judging from our experiments the gangliar mechanism gives the dilator effects more easily than does the peripheral mechanism. That may be due to a partial masking of the dilator mechanism by the constrictor mechanism in the latter region.

In regard to the peripheral effect there seems to be a distinct difference between the limb reaction and that of the spleen. In the perfused limb small amounts of adrenalin injected into the perfusion fluid cause pure dilatation, larger amounts may cause dilatation followed by constriction, while very large doses may cause pure constriction. On the other hand in the perfused spleen if dilatation is obtainable from adrenalin injected into the perfusion fluid, it follows constriction, at least in our experience.

## SUMMARY

1. Dilatation of the spleen is caused by the action of adrenalin upon the twelfth and thirteenth dorsal root ganglia, the semilunar ganglion or upon some terminal structure in the spleen itself.

2. Constriction from adrenalin can result from the response of a mechanism in the dorsal root ganglia or from a structure in the spleen.

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# THE ACTION OF ADRENALIN ON THE KIDNEY

Frank A. Hartman and Ross S. Lang.

(From the Department of Physiology, University of Toronto)

Many investigators have studied the action of adrenalin on the kidney, both in regard to circulatory changes and to urine flow, and have found that one or both may be modified by this substance. Inasmuch as Cow (1) has shown that there is direct communication between the adrenal medulla and certain parts of the kidney, it appears that adrenalin might have some important function in the control of the kidney. In the present instance we have made a study of the influence of adrenalin on the kidney volume, both from gangliar and peripheral action. Although it is possible that adrenalin may influence urinary secretion independent of vascular changes, yet we know that if vascular changes occur they will also modify kidney activity. It is assumed that volume changes are due to vascular changes.

## METHODS.

The methods employed were similar to those used in a previous study of the spleen (2), the kidney being enclosed in a gutta percha oncometer which was connected with a Brodie bellows recorder.

In the perfusions the vessels were all tied off and warm oxygenated Ringer's solution forced into the renal artery under a constant pressure. Injections of adrenalin into the perfusion fluid were made at the entrance of the perfusion cannula by means of a hypodermic needle piercing the rubber tubing. Passive effects of the injection were ruled out either by slow injection or else by a simultaneous removal of an equal quantity of perfusion fluid by another needle inserted farther back in the connecting rubber tube.

All animals were under the influence of ether. Adrenalin solutions were made by diluting Parke, Davis & Co.'s adrenalin chloride solution with distilled water.

## RESULTS.

In an earlier research (3) we found that small doses of adrenalin injected into the general circulation caused constriction of the kidney, while in some instances larger doses caused

constriction followed by dilatation. Brief dilatation preceding constriction occurred at times, but appeared to be a passive result from a short rise in blood pressure.

Five more cats and three dogs were studied in this way, with results which agree with the earlier research.

One experiment may be cited. The kidney of a dog weighing 18 kgm. responded by constriction to doses of adrenalin ranging from 0.2 cc., 1:100,000 to 0.4 cc., 1:10,000. These were all depressor doses of adrenalin. The response to doses ranging from 0.4 cc., 1:10,000 to 3.0 cc., 1:10,000 was constriction followed by dilatation (Fig. 1); 0.6 cc., 1:10,000 was a depressor dose, while 1.3 cc. of the same dilution was pressor in effect.

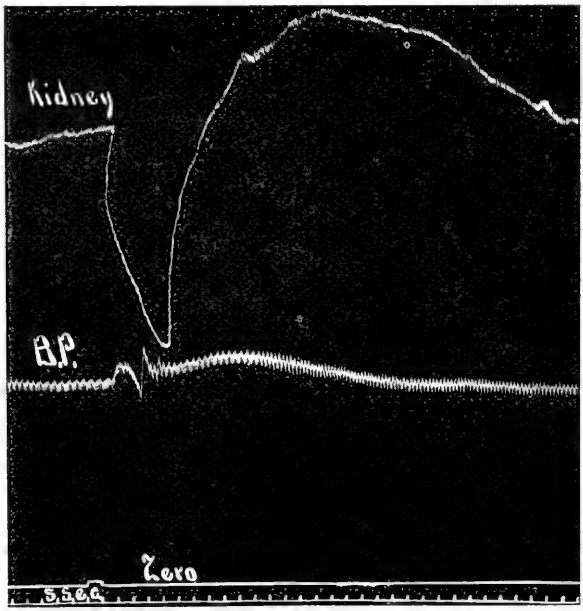


Fig. 1. Constriction and dilatation of a normal kidney from adrenalin, 1.3 c.c., 1:10,000 injected into the jugular vein. Dog 18 kgm.

Although this delayed dilatation occurring in the kidney was similar to that occurring in the intestines (3, p. 313), with large doses of adrenalin it was by no means so prevalent. However, in those individuals in which it was obtained it resulted repeatedly from injections above a certain dose.

We next attempted to locate the regions where adrenalin could produce these two effects, i. e., constriction and dilatation. In order to separate peripheral from gangliar or more central effects, we completely cut off the kidney from the body circulation, then perfused it. Nervous connections to the kidney were carefully preserved in the operation. Both kidneys were perfused alternately in two dogs. The first was an animal (18 kgm.) that gave constriction followed by dilatation of the kidney when its circulation was intact and a large dose of adrenalin was injected into the jugular vein. When perfused, the left kidney gave dilatations from jugular vein injections of doses above 0.2 cc., 1:10,000. Sometimes slight constriction preceded the dilatation (Fig. 2). Injections of adrenalin into the perfusion

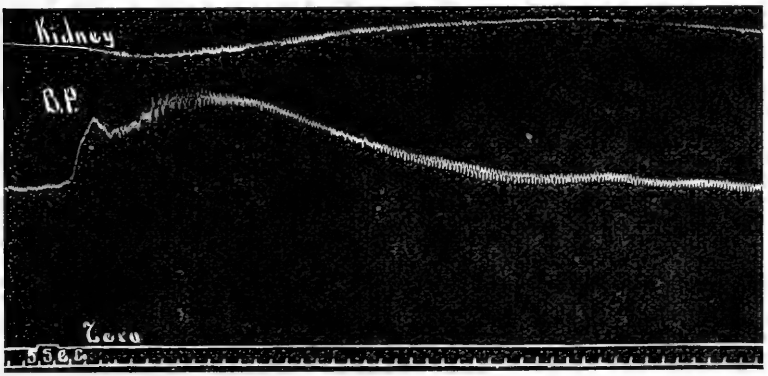


Fig. 2. Constriction and dilatation of a perfused kidney, 2 c.c., 1:10,000 adrenalin injected into jugular vein. Dog 18 kgm.

fluid caused a similar effect, i. e., constriction followed by dilatation (Fig. 3). Occasionally the dilatation was followed by constriction. The other kidney responded in a similar manner, both before and after perfusion.

The second dog (15 kgm.) gave dilatation in both perfused kidneys from adrenalin injected into the jugular vein, while injections into the perfusion fluid caused constriction (Fig. 4). Doses as small as 0.2 cc., 1:100,000 gave this result.

Volume changes in perfused kidneys from jugular vein injections of adrenalin, may be due to action on structures in the semilunar ganglion, dorsal root ganglia or in some more central location. We tried the effect of direct application of adrenalin to these

ganglia. The ganglia were usually slit to facilitate absorption. In the case of the semilunar ganglion, the mesentery was cut and separated from it in such a way that a pocket could be made

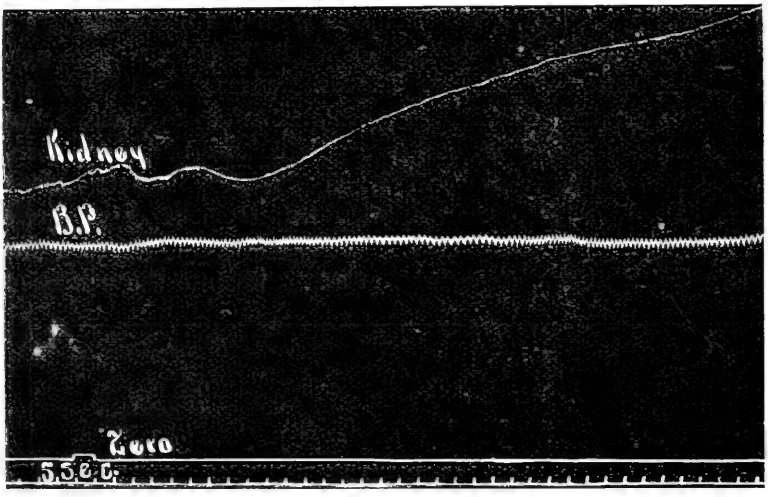


Fig 3. Constriction and dilatation of a perfused kidney from the injection of 0.2 c.c., 1:100,000 adrenalin into the perfusion fluid. Dog 18 kgm.

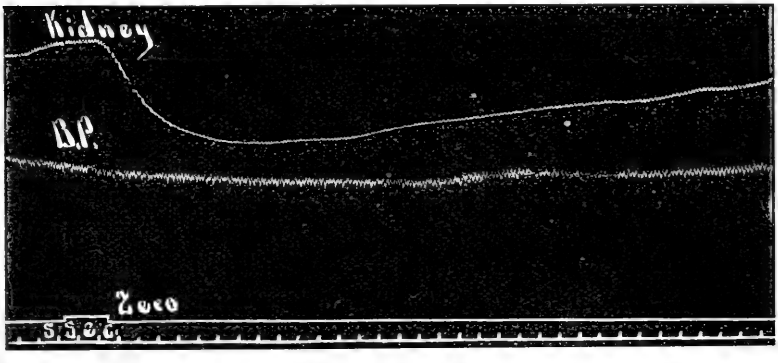


Fig. 4. Constriction of a perfused kidney from the injection of 1.3 c.c., 1:100,000 adrenalin into the perfusion fluid. Dog 15 kgm.

by engaging the cut surface of the mesentery with haemostats. Adrenalin solutions could then be confined in this pocket without absorption into the general circulation.

Adrenalin action on the semilunar ganglion was studied in three cats and one dog. Dilatation of the kidney was obtained in all of these when adrenalin was applied to the ganglion in question. In some animals, concentrations as low as 1:100,000 produced this result; in others a 1:10,000 solution was necessary (Fig. 5). In two of the cats the latter solution sometimes caused

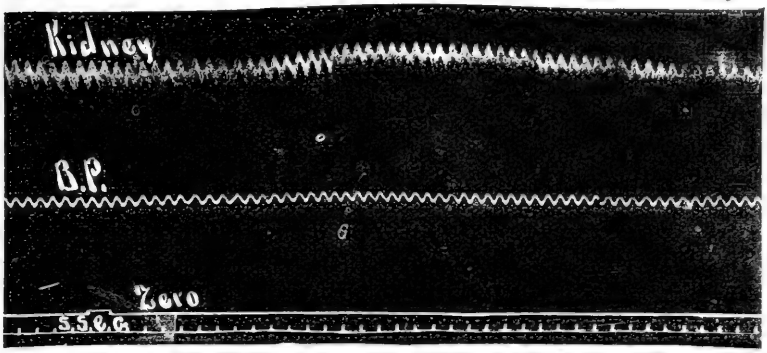


Fig. 5. Dilatation of the kidney caused by the application of 1:10,000 adrenalin to the semilunar ganglion. Cat. 3.1 kgm.

dilatation followed by constriction. This could be explained on the ground that small amounts of absorbed adrenalin affect the dilator mechanism, while larger amounts bring the constrictor mechanism into action. This was confirmed by the pure constriction which it was possible to obtain with concentrated adrenalin solutions (1:1,000) (Fig. 6).

We concluded from these observations that adrenalin can influence the volume of the kidney by action upon both dilator and constrictor mechanisms located in the semilunar ganglion, the result depending upon the concentration of adrenalin absorbed.

The effect of adrenalin through the dorsal root ganglia was studied in four cats. With the animal lying on its side, an opening extending transversely from the midline was made in the abdominal wall above the kidney. The kidney was placed in the oncometer and the apparatus properly adjusted before exposure of the dorsal root ganglia. The twelfth and thirteenth thoracic ganglia were carefully exposed and their connections with the spinal cord severed. After allowing a short time for the bleeding to stop, the adrenalin solution was applied to a ganglion. In

some cases, to make sure that adrenalin was not escaping into the general circulation, the ganglion was surrounded by rubber dam. The earlier the adrenalin was applied the more sensitive was the ganglion. In fact, if the ganglion had been exposed too long or the blood pressure had become extremely low, there was

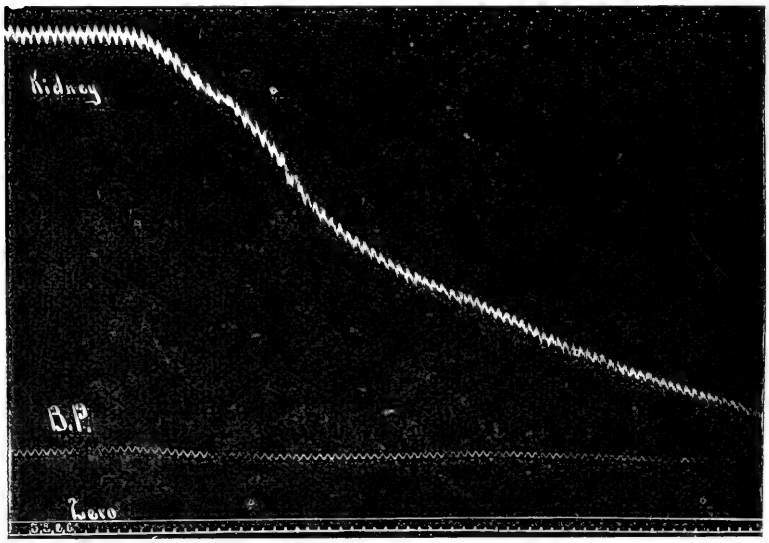


Fig. 6. Constriction of the kidney caused by the application of 1:1,000 adrenalin to the semilunar ganglion.

either no response or else only a slight effect. Second and third applications to the same ganglion had no effect unless several minutes intervened and the ganglion was thoroughly washed with isotonic salt solution. The adrenalin solution was warmed to 37° C. because cold solutions of distilled water sometimes produced an effect.

In one animal, constriction of the kidney was produced by 1:10,000 adrenalin applied to the dorsal root ganglia. No dilatation was obtained. The blood pressure, however, was quite low (32 mm.).

Dilatation of the kidney was produced in the three remaining animals from solutions of 1:10,000. One of these animals gave a similar response with 1:100,000 adrenalin. The response is frequently very slow, due no doubt to the slow absorption by the ganglion (Fig. 7).

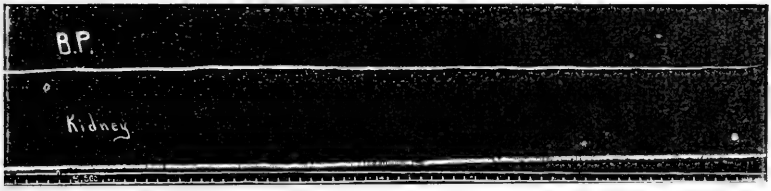


Fig. 7. Dilatation of the kidney produced by painting a dorsal root ganglion with 1:10,100 adrenalin. The ganglion was surrounded by rubber dam. Cat 2.4 kgm.

## DISCUSSION.

Our experiments prove that adrenalin frequently causes dilatation of the kidney. This dilatation can be caused by action on the semilunar ganglion, dorsal root ganglia, or, in some cases, on structures in the kidney itself.

Hoskins and Gunning (4) obtained dilatation following constriction in one dog out of sixteen from intravenous doses. This has been more frequent in our experiments, as five out of nine gave this response. In addition to these experiments, which were upon kidneys with an intact circulation, we have obtained dilatation of the perfused kidneys of two dogs.

Kidney dilatation from small doses of adrenalin may be more common than one might suppose. However, the constrictor mechanism in the kidney tends to predominate in adrenalin responses.

In view of the recent work of Addis, Barnett and Shevky (5) we tried to obtain dilatation of the kidney in a rabbit by the application of adrenalin to the semilunar ganglion. Concentrations of adrenalin from 1:100,000 to 1:10,000 caused only constriction in the kidney. This is confirmatory of recent work from this laboratory (6), which has shown that rodents are exceptional among mammals in that adrenalin vasodilator mechanisms are either absent or else insignificant in their action.

We also attempted to produce volume changes in the kidneys of cats by subcutaneous injection of adrenalin. Doses of 0.5 cc., 1:1,000 produced no distinct result. Three animals were tested in this way. Therefore, it seems that even in animals which are known to possess adrenalin vasodilator mechanisms subcutaneous injections have little effect upon the volume of the kidney.

In regard to the effect of adrenalin mingled with the perfusion fluid fed to a kidney, numerous observations have been made by others. Sollmann (7), with relatively large doses of adrenalin, obtained constriction. He says, however, that after several hours' perfusion, or sometimes earlier, the constrictor action disappears and that at times it is replaced by a dilator action. Pari (8) obtained one case of dilatation from adrenalin in the perfused kidney.

SUMMARY.

1. Adrenalin in moderate amounts produces dilatation of the kidney in some individuals.
2. Dilatation is usually preceded by a brief constriction.
3. Adrenalin can produce dilatation by its action on either the semilunar ganglion, dorsal root ganglia, or on some structure in the kidney.
4. Likewise constriction can be produced by adrenalin acting either in the semilunar ganglion, dorsal root ganglia, or the constrictor structures in the kidney.

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# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES

No. 29: SOME RECENT WORK ON THE CONTROL OF THE RESPIRATORY CENTRE, BY J. J. R. MACLEOD

(REPRINTED FROM THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, VOL. V)


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# SOME RECENT WORK ON THE CONTROL OF THE RESPIRATORY CENTER

BY

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Reprint of Editorial from

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## *Some Recent Work on the Control of the Respiratory Center*

FROM the moment the animal is born until death, breathing proceeds with a rhythm which is occasionally broken for brief periods of time by voluntary holding of the breath or by participation of the respiratory musculature in the various expulsive acts of the body, or in phonation and singing. The respiratory movements involve the harmonious activities of greatly diverse muscular groups, some of them contracting, while others relax, but always in so perfect a synchronism that the movement produced alters the capacity of the thorax in the manner which will most effectively ventilate the pulmonary alveoli. During inspiration, for example, the muscles which elevate the thoracic cage and those which depress the diaphragm contract at the same time that the muscles of the abdominal walls relax to make more room for the depressed viscera.

The excitatory or inhibitory nerve impulses which control these movements come finally, of course, from the cells of the lower neurones and these are scattered along the cerebrospinal axis from the level of the nerve centers for the muscles of the alæ nasi in the pons to those of the abdominal muscles in the lumbar region of the spinal cord. But it is plain that these centers can not in themselves be more than local executives for a higher command which must have its headquarters in some more or less localized group of nerve cells. This chief respiratory center, as it is called, is usually considered to be situated in the medulla oblongata but there is good reason for believing that its upper limits extend for some distance into and perhaps beyond the pons.

It is clear that the fundamental problems of respiratory control must be directed to ascertain the conditions which excite or alter the activity of this center, and it is around this question that much important work has been contributed during recent years, particularly by the Oxford School of Physiologists led by J. S. Haldane[1] and the Copenhagen School led by August Krogh.

There are in general two ways in which the activities of a center might be caused to alter. These are by changes in the chemical composition of the blood supplying it and by nerve impulses derived from other parts of the nervous system.

Confining our attention for the present to the former class of influences, it may be said that tendency is to consider the hydrogen-ion concentration ($C_H$) of the blood that bathes the center as chiefly responsible. According to this view alterations in $C_H$ furnish the respiratory hormone.

While there can be no doubt that the respiratory center is extremely sensitive to the slightest changes in $C_H$ of the blood, indeed, it is probably safe to say that there is no more sensitive indicator of changes in $C_H$ than the respiratory center, yet many serious objections can be raised against the view that the ordinary physiologic alterations in respiratory activity are brought about in this way. Because of the fact that the center is sensitive to changes in $C_H$ which can not be measured by any known laboratory method it is impossible to furnish direct proof for or against the hypothesis. All the evidence is of an indirect

nature and in many cases it is dependent upon assumptions and analogies which may possibly be erroneous.

It has long been known that respiratory activity can be excited by experimentally raising $C_H$ of the blood, through the injection of mineral acids intravenously, but this does not necessarily mean that ordinary (physiologic) alterations in that function are due to the same cause. A great part of the indirect evidence is based on the observation that the tension of carbon dioxide ($CO_2$) in the blood bears a relationship to $C_H$ of this fluid and to the degree of pulmonary ventilation. This is the case because $CO_2$ in solution is a weak acid and therefore cooperates with the other acids of blood to maintain $C_H$. It possesses one advantage over the other acids in that it is volatile and consequently can readily be got rid of through the alveoli. Whenever, therefore, there tends to be an increase in $C_H$ of the blood some of the $CO_2$ which is in simple solution in the plasma is got rid of so that the tension declines and the percentage of $CO_2$ in the alveolar air becomes lower. The tension of $CO_2$, in other words, declines so as to make room in the blood for other acids. When the adjustment is perfect, the condition is often called *compensated acidosis,* but when there is too much fixed acid, so that $C_H$ is slightly raised, it is called *uncompensated acidosis.* In the former case there is no respiratory disturbance when the person is at rest, but such is readily induced by the slightest exertion because there is a deficiency of basic substance available in the blood and tissues to combine with the increased $CO_2$, and other acids, produced by the active muscles; the *buffer action* of the blood is said to be depressed. In the latter case, on the other hand, there is hyperpnea even at rest.

The H-ion concentration may obviously be raised by a process which is fundamentally the reverse of that just considered; namely, by an increase in the $CO_2$ tension while the other (fixed) acids of the blood remain constant. In this case acidosis will occur and there will be hyperpnea along with a higher percentage of $CO_2$ in the alveolar air; this condition is styled carbonic acid[2] and it occurs in uncompensated cardiac cases and to a certain extent in asphyxial conditions and during strenuous muscular exertion.

In all of the foregoing instances the interpretation of the respiratory excitement which has almost universally been adopted is that $C_H$ of the blood has become raised, but if we pause to consider all the facts, it will be seen that the conclusion is by no means inevitable. It may as well be that it is the free $CO_2$ as such, or more precisely the anion $HCO'_3$ (for $H_2CO'_3$ will dissociate into $H^{\cdot} \rightarrow HCO'_3$) that is the really important hormone instead of the cation $H^{\cdot}$. In the cases of carbonic acid acidosis this is easy to understand; in cases of uncompensated acidosis it may be explained if we remember that there is now no sufficient amount of base to take up and fix as carbonates the $CO_2$ as it is produced, so that the free $CO_2$ in the cells of the respiratory center, as in other cells, is not adequately removed. There is a certain amount of experimental support for this view of which the following may be cited: Hooker, Wilson and Connett[3] succeeded in retarding death in the basal regions of the brain sufficiently so that respiratory movements were still present and they found that these movements became more markedly excited at a certain $C_H$ of the perfusion fluid when the acid present was mainly $H_2CO_3$ than when it was any other acid. R. W. Scott[4]

found that the respiratory movements in decerebrate cats were increased in proportion as the percentage of $CO_2$ in the respired air was raised. Examination of the arterial blood by the colorimetric method showed that $C_H$ also became increased under these conditions, an observation which in itself, might support the view that it is really elevation of $C_H$ that furnishes the stimulus to the respiratory center. That this is not the sole, if even the main, stimulus was shown in further experiments in which amounts of alkali were first of all injected intravenously so that decided depression in $C_H$ of the blood was established. On now causing these "alkalosis" animals to respire in $CO_2$-rich atmospheres it was found that the respirations were excited practically to as great a degree as in the animals with normal $C_H$; although this became raised, it had not nearly attained the level at which it stands in normal blood even when very marked hyperpnea was present. Increase in $CO_2$ tension, quite independently of increase in $C_H$ above the physiologic level, had quite clearly afforded the stimulus to the center. It will be necessary to repeat the observations by the use of the electrometric method for measuring $C_H$ of the blood.

If further investigation should confirm the hypothesis that $CO_2$ tension is a more effective stimulus for the respiratory center than $C_H$ it will mean that, unlike the rhythmic action of the heart which is highly susceptible to cations the rhythmic action of the respiratory center is so to anions. The excitability of the respiration center towards certain concentrations of the CN-ion is of great interest and significance in this connection although the action is differently explained by its discoverer, Loevenhart. The rhythmic contractions of the isolated small intestine are also highly susceptible to the influence of anions.

Apart from their theoretical interest the foregoing facts are of undoubted practical importance since they show that a certain tension of $CO_2$ must exist in the blood which bathes the respiratory, and very likely other centers, in order that the physiologic activity may be maintained. The observations recall the old hypothesis of Mosso that certain perversions of physiologic function may occur when the tension of $CO_2$ is subnormal (acapnia).

One of the most important questions in connection with the hormone control of the respiratory center concerns the influence of a deficiency of oxygen in the inspired air. It has long been known that dyspnea usually supervenes in atmospheres which are decidedly deficient in this gas. There are two well-known types of observation which illustrate this fact. The first of these is the laboratory experiment in which a person is caused to breathe in and out of a large spirometer or rubber bag provided with soda lime to absorb carbon dioxide, dyspnea develops, becoming very marked when the oxygen has fallen below 14 per cent. The other is afforded by watching the respiration at high altitudes, it is hyperpneic so that the alveoli are more thoroughly ventilated and the supply of oxygen in them becomes more frequently replenished in order to compensate for the decreased percentage in the atmosphere. There is therefore no doubt that deficiency of oxygen excites the respiration; the question is whether the stimulus is the oxygen deficiency *per se* or whether it is dependent upon some condition which is set up by this deficiency. In considering this question it is important to distinguish between extreme and moderate degrees of oxygen deficiency.

When it is extreme the respiratory center, like all other centers, becomes depressed apparently without any preliminary stimulation, and breathing ceases. Such a condition occurs when the blood supply to the medulla is seriously interfered with and in cases of respiration in poisonous gases, like carbon monoxide or methane. A similar depression of the respiratory center due to oxygen deficiency may possibly be the cause of death in such diseases as acute pneumonia and edema of the lungs. It is of decided practical value to know that it is possible to restore a center rendered inactive through deficiency of oxygen by increasing the percentage of this gas in simple solution in the blood supplying the medulla. We have observed this restorative power of oxygen inhalations very strikingly in the case of decerebrate cats (Fraser, Lang and Macleod). These animals breathe with perfect regularity as long as there is an adequate supply of oxygen to the medulla, but if this be curtailed, as by temporarily clamping the vertebral arterioles, the respirations gradually cease but return immediately the circulation is restored. Sometimes, especially when the arterio corpora quadrigemina are destroyed, the breathing in the decerebrate animals becomes irregular and gradually ceases entirely, though the heart is still beating and there is a fair arterial blood pressure. In such cases normal respiration is promptly restored by raising the partial pressure of oxygen in the alveolar air which is most conveniently done by introducing pure oxygen low down in the trachea through a catheter and interrupting the stream rhythmically at about the same rate as the animal breathes. The restored breathing continues for some time after discontinuing the oxygen inhalations.

In view of the results it is possible to explain the beneficial effects which often follow the administration of oxygen in cases of pneumonia, in coal gas poisoning, etc. The inhaled oxygen raises the tension of this gas in the alveolar air so that a sufficient amount of it becomes dissolved in the plasma to keep the center alive, independently—in the case of CO-poisoning at least—of the formation of more oxyhemoglobin. That nerve centers and other tissues can be kept alive by physiologic saline in which excess of oxygen is dissolved is a well-established fact and it should be our aim, when treating cases of asphyxia, to raise the partial pressure of this gas in the alveolar air as high as possible. The dissolved oxygen supplied to the centers in this way must be maintained until the mechanism of which the supply is normally ensured, namely, by dissociation of the oxygen bound to hemoglobin, has been restored to normal. The resuscitation afforded by increasing the percentage of oxygen in the alveolar air, although it can only be temporary, may serve to tide over a crisis and so permit the normal mechanisms by which oxygen is transported to the tissues to become restored.

With regard to the second method of respiratory control, namely, that through afferent nerve impulses only a few of the most outstanding facts can be referred to here. The older work seemed to show the most important of these impulses to be transmitted to the center along the vagus nerves and the hypothesis was formulated that the rate of the respiratory movements depends fundamentally on the fact that towards the end of each inspiration an impulse set up by the distention of the alveoli, is transmitted to the center where it inhibits the rhythmic discharge and so brings on an expiration. Without these inspiration

inhibitory impulses respiration is much slower and deeper than normal. There is a growing mass of evidence which goes to show that these afferent impulses, as well as others derived from the afferent nerves of the thoracic parietes (including muscular sense impressions) are important in harmonizing the action of the respiratory musculature much in the same way that afferent impulses from the extremities are important in the synthesis of the complicated muscular activities necessary for the maintenance of the erect posture and for locomotion (Pike,[5] Boothby and Berry[6]).

That the respiratory center is influenced by afferent impulses which are set up by the degree of distention of the lungs has been shown in experiments by Lois Fraser, Lang and Macleod.[7] The experiments were performed on decerebrate cats. When these animals were caused to breathe into wide-bore tubing provided with bottles containing soda lime to absorb the carbon dioxide it was found that the respiratory volume became markedly increased while there was still practically no reduction in the percentage of oxygen in the inspired air. This evidence, furnished by registration of the volume of respired air (by a Gad-Krogh spirometer) was confirmed by observing the behavior of the respiratory quotient of the alveolar air. Immediately the breathing into the tubing was started the quotient rose considerably, sometimes to 2.0, indicating that $CO_2$ was being washed out of the blood by the more thorough ventilation of the alveoli. When the animal was allowed to breathe in outside air again the breathing respiratory volume quickly returned to the normal and the respiratory quotient fell to a very low level showing that the blood was now taking up the $CO_2$ it had lost.

This experiment recalls the experience of every one who has tried to breathe through tubing into a spirometer or gas absorbing apparatus; a certain degree of hyperpnea is always set up which is usually attributed to a conscious sense of effort. But the foregoing observations show clearly that the reaction is independent of the higher centers and that it must be purely reflex through the respiratory center, the afferent stimulus being the state of distention of the lungs or thorax. The stimulus which excites the hyperpnea may persist indefinitely or it may subside after a time. In cases in which it does not subside it will lead to an overventilation of the alveoli and consequently to a depletion of the free $CO_2$ of the blood (acapnia). It is possible that it is because of this condition that prolonged respiration through a gas mask or into a respiratory apparatus frequently becomes unbearable on account of the sense of bodily discomfort which develops (mask staleness).

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—*J. J. R. M.*

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STUDIES

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# Studies in the Regeneration of Denervated Mammalian Muscle*

1. Volume Changes and Temperature Changes
2. Effect of Massage

Approved for Publication by J. T. Fotheringham, Major-General, C.M.G.,
Acting Director-General of Medical Services
Ottawa, Canada, May 27, 1919

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# STUDIES IN THE REGENERATION OF DENERVATED MAMMALIAN MUSCLE.

## I. VOLUME CHANGES AND TEMPERATURE CHANGES.

By F. A. Hartman, S/Sgt. W. E. Blatz and L. G. Kilborn.

*(From the Laboratories of the Military School of Orthopædic Surgery and Physiotherapy, Hart House, and of the Department of Physiology, University of Toronto, Toronto, Canada.)*

In conjunction with a study of the influence of treatment on denervated muscle we have carried out investigations concerning the circulatory changes which take place in it.

### I. Volume Changes.

One of the immediate results which is known to occur after severing the nerve to a muscle is a dilatation of the blood vessels thus cut off from the nerve supply. This leads to an increase in the volume of the limb. We have studied these volume changes by means of the plethysmograph.

*Methods.*—Cats were the subjects for these experiments. They were anæsthetised with urethane except in one experiment where ether was employed. Volume changes were studied in one or both hind limbs, when both were used one served as a control. The limb was enclosed either in a glass or tin cylinder, which was made air-tight by packing a vaseline-cotton-wool mixture between the thigh and cylinder mouth. The cylinder was connected to a Brodie bellows of such a capacity that it would be sure to accommodate the volume changes in the limb. The experiments were performed in a "constant" temperature room which was devised by the late Professor Brodie. Records were kept of the temperatures of both the room and air within the plethysmograph. Loss of heat from the plethysmograph was prevented by a thick wrapping of cotton-wool.

The sciatic and femoral nerves, in the limb to be denervated, were prepared for sectioning before fitting the plethysmographs in place. The former nerve was exposed just after it left the sciatic notch, and the latter where it emerged from under Poupart's ligament. A ligature was passed under the femoral nerve so that it could be lifted for cutting when desired. This method was not satisfactory in the case of the sciatic nerve because when the plethysmo-

graph was in place the nerve could not be cut without pulling, nor could the plethysmograph be disturbed. In order to discover how much the stretching of the nerve might vitiate the results, the sciatic nerve was merely pulled after the limb had been enclosed in a plethysmograph. The volume change was almost as much as in a denervated limb. Of course the tension was greater than that which accompanies the preparation of the nerve for cutting. But it serves to demonstrate the care needed in preparing the nerves for cutting in these experiments. Therefore an instrument was devised which could be put in place so that the nerve could be cut at any time without moving the plethysmograph or stretching the nerve.

The neurotome (Fig. 1) is a small tube (*C*) plugged at the lower end (*a*). A hole (*b*) large enough to receive the nerve is cut in the

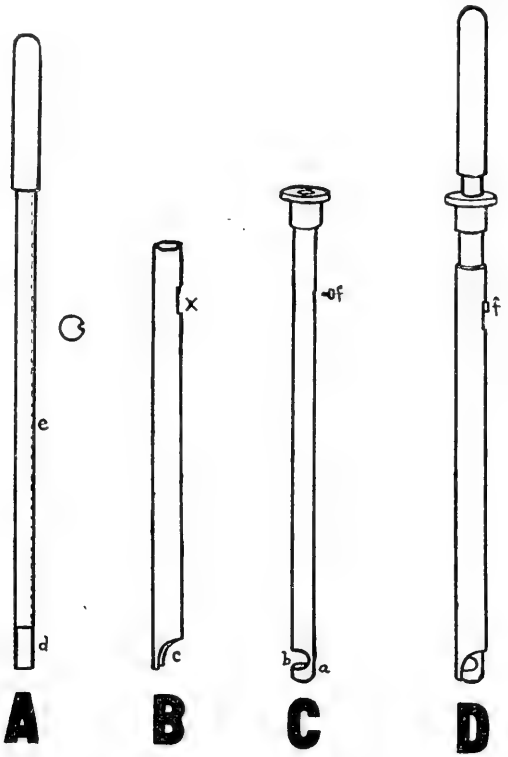


Fig. 1. Neurotome. (See Text.)

side. The escape of the nerve when in position is prevented by a well-fitting outer cylinder (*B*) with a projecting tongue (*c*), which is pushed into place from above (see *D*). The knife (*A*) is shaped like a chisel and is as wide as the internal diameter of the inner cylinder. This chisel (*d*) is fastened at the end of a brass rod (*e*) which fits snugly inside the inner tube. A bed for the chisel to work against is furnished by making a slot in the plug (*a*) at the lower end of the tube, to receive the cutting edge. A small screw (*f*) fastened in one side of tube (*C*) projects into a groove (*e*) in *A*. This prevents turning of the chisel. The same screw holds outer tube *B* in position, not only preventing rotation, but limiting its movement by slot *X*. (*D*) shows the instrument assembled.

After the nerves had been prepared for sectioning, the cylinder was put in place, and a record started on a slowly-moving kymograph. After the lapse of 30 to 60 minutes, when we were certain that the limb volume was constant, the nerves were cut. Records of the room temperature, rectal temperature, and of the air in the plethysmograph were taken every half hour in the early part of the experiment, and later every hour.

*Results.*—A cat under ether served as the first subject. Blood pressure from the carotid artery was registered every 30 minutes. The blood pressure fluctuated from 107 mm. to 128 mm. during the first five hours after denervation; then it gradually fell until it was 81 mm. at the seventh hour. The animal died at the end of eight hours. Maximum dilatation was reached five and one-half hours after denervation.

In a second cat under urethane the maximum dilatation was reached six hours after denervation. The blood pressure showed little change until the ninth hour where from a pressure of 92 mm. it gradually fell to 63 mm. by the end of the thirtieth hour. Unfortunately the temperature of the room was not controlled, so that a reduction in volume during the latter hours of the experiment cannot be taken as of any great significance.

Two more experiments were performed, in which changes only in the denervated limb were studied. In the first the maximum dilatation occurred 5·7 hrs. after denervation. This was one-half hour after the maximum temperature of the air within the plethysmograph was reached. These changes were independent of the room and rectal temperatures after the third hour. In the second animal, the maximum dilatation had developed at the end of the second hour. And then after an hour of little change, there was a fairly steady reduction in volume, especially pronounced during the succeeding six hours. This decrease in volume was too great to be accounted for

entirely by the decrease in rectal and plethysmographic temperature (see Fig. 2). By the end of the seventeenth hour the plethysmo-

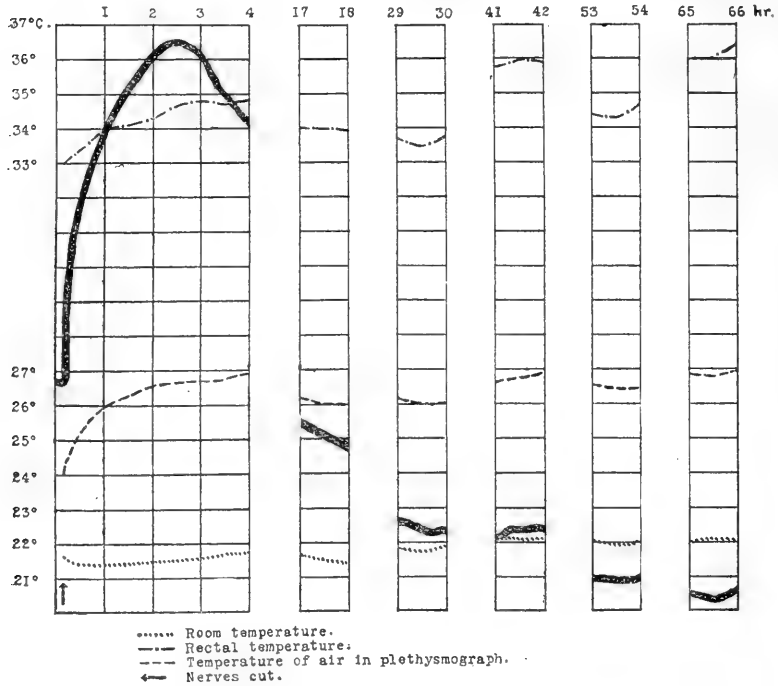


Fig. 2. Volume change in denervated hind limb. Heavy line represents limb volume.

graphic volume had returned to a little below normal. However, this was in part due to a fall in temperature of air within the plethysmograph. But by the forty-first hour there was a decided reduction in the volume of the limb which was independent of a reduction in air temperature within the plethysmograph because that temperature had again returned to its former height. This reduction of the limb volume continued to increase although the temperatures were not decreased. This experiment would seem to indicate that there is an over-recovery of the volume of the limb after dilatation and that this may be independent of a lowering of temperature in the limb. Indeed it appears that the limb temperature may remain as high as at the time of maximum dilatation. The bellows did not leak, and the animal remained in good condition, as indicated by the blood pressure,

until the experiment was stopped (75 hrs.). The blood pressure was 114 mm. at that time. The animal had recovered sufficiently from the urethane to give the corneal reflex.

In an attempt to secure better evidence of an over-recovery of the volume of the denervated limb, volume changes of both normal and denervated limbs were studied in three cats.

Unfortunately in the first experiment, the room temperature was allowed to drop after the seventh hour, so that constriction in the bellows took place as a direct result of this fall in temperature of the external air. However, the maximum dilatation had been reached at

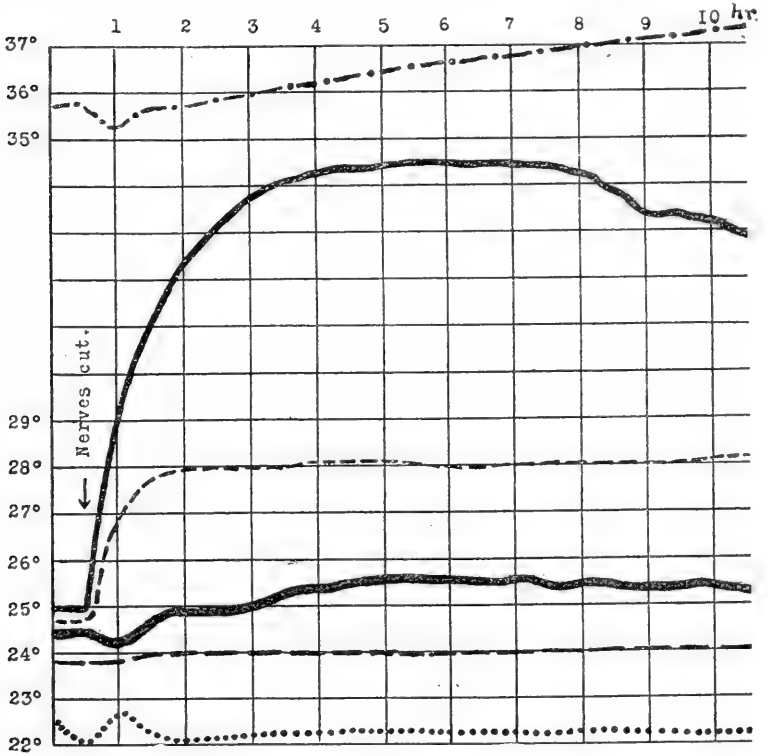


Fig. 3. Volume change in a denervated limb. Slower recovery than in Fig. 2. Upper heavy line, volume of denervated hind limb. Lower heavy line, volume of normal limb.

the fourth hour and the change was reversed within a few minutes, so that a steady constriction ensued. That this constriction was independent of any external influence, at least up to the ninth hour, was shown by the maintenance of the temperature of the air within the plethysmographs surrounding both limbs; moreover the normal limb did not constrict until the tenth hour. At the ninth hour the denervated limb had recovered its normal volume, as indicated by the bellows. Therefore this experiment tends to confirm the observation in the preceding experiment, that dilatation from denervation is recovered from in the course of several hours. The temperature within the plethysmograph surrounding the denervated limb did not begin to fall until four hours after the constriction had commenced. This also confirms the result in the previous experiment.

In the next experiment the room temperature was carefully controlled. Here again constriction of the denervated limb occurred unaccompanied by any fall of temperature of the limb as indicated by the air in the plethysmograph (Fig. 3). The maximum dilatation was reached in five hours, while the maximum temperature rise of the plethysmographic air had occurred in about two hours.

These results were further confirmed by a third experiment lasting twenty hours. In this, maximum dilatation was reached in two and one-half hours. From that time constriction took place, although so slowly that the normal volume had not been recovered until twenty hours after denervation. The temperature within the plethysmograph, however, did not fall until the fifteenth hour.

We next attempted to get some idea of the quantitative change in the volume of a limb after denervation. In order to reduce the amount of air which might expand from the increased temperature of the limb, the cylinders were filled with cotton-seed oil after being carefully fitted to the limbs, merely the tube and bellows and a small space near the opening of the tube containing air. At the end of the experiment, immediately after the death of the animal, the temperature of the oil was raised by external heat, then by noting the temperature increase, together with the bellows changes, we could tell approximately the volume increase for each increase of a degree. By subtracting the expansion which would be due to rise of temperature of the air in the plethysmograph during the experiment the volume increase due to expansion of the limb was estimated. According to this method the limb actually expanded 2·7 c.c. The volume of the limb was determined by displacing water from a cylinder. This method of course could not be accurate, but it gave a fair idea of the magnitude involved. The denervated limb was found in this manner to be 105 c.c. in volume. The percentage expansion then was about 2·5.

*Discussion.*

Gaskell (1) reached the conclusion that the increased blood flow due to denervation was very transient in character. He studied the venous flow from the extensor vein in dogs. The maximum increase in flow was attained in 20 to 40 seconds. The increase disappeared in from two to four minutes, and it amounted to as much as nine to eighteen times the normal. He showed that the quick recovery to the normal rate of flow was not due to loss of blood, because if he waited for a few minutes after cutting the nerve before the blood was permitted to escape, the rate of flow was normal.

Goltz (2) believed that the dilatation resulting from nerve section was due to excitation of vasodilators. If that is correct rather quick recovery of the vessels should be expected as the effect of stimulation would not last long.

Our observations are based on the volume change which may or may not indicate a change in rate of flow. In view of Gaskell's work the most plausible explanation of our result is that following the transient dilatation and resulting increase in flow, there is constriction at some region such as the arterioles, capillaries or venules, while the other vessels continue to dilate or remain in an expanded condition. Thus the rate of flow might be quickly reduced to normal, but due to the congestion the limb remains dilated. Then as time went on the congested vessels gradually recovered their former size, so that in the course of many hours the congestion or dilatation would disappear. If this is the correct interpretation, the increase in volume does not indicate an increased circulation to the limb. In fact the rate of flow might actually decrease.

On the other hand such an interpretation would not account for the prolonged rise in temperature which has been observed by many investigators (2, 3). This must be due to either increased circulation or else to a local increase in heat production. As far as we know, heat production in muscle is associated with contraction. Therefore increase in tone or contraction would be necessary to account for such a condition. The only indications of increased muscular activity are the observations by Schiff (4) and others that fibrillation occurs in denervated muscle. However, Langley, and Kato (5) did not observe fibrillation until four days after nerve section. This makes a considerable gap during which fibrillation apparently could not account for a local temperature increase.

The increase in volume together with the increase in temperature seem to indicate a prolonged circulatory increase after nerve section. We are unable at present to reconcile this view with Gaskell's work.

## II. Temperature Changes.

It is well known that the temperature of a muscle is increased after denervation. Goltz (2) studied the change in skin temperature in a denervated limb. He found that the increased temperature persisted from ten to twenty-eight days. His method, however, did not permit very exact observations. Heidenhain (3) observed the temperature of denervated muscle by means of a very sensitive thermo-electric couple. He came to the conclusion that the rise of temperature often lasted for weeks. Many others (1, 6) have found similar results.

We have tried to follow the temperature changes over longer periods of time than was done by the earlier workers. For example, where they made observations continuously for but a few hours after denervation, we have done so for two or three days in some cases.

*Methods.*—Cats were used as in the volume experiments. They were placed under the influence of urethane in experiments of long duration, but when only one set of readings was desired ether was employed. The animal was protected from loss of heat by covering with cotton-wool and by carrying on the experiment in an unusually warm room. Sometimes artificial heat was supplied by means of an electric heater arranged to reflect upon the animal. Many of the experiments were conducted in a "constant" temperature room. Artificial heat was furnished by 8 c.p. carbon lamps located in different parts of the room near the floor. These lamps were turned on or off as the temperature showed a tendency to vary. The room was ventilated by an electric fan. Undue loss of heat from opening the door was prevented by a vestibule and second door.

The femoral and sciatic nerves were exposed and cut with a neurotome as described in § I.

We used very sensitive mercury thermometers which registered from—5° to 50° C. They were graduated in tenths of a degree and each division was great enough (each degree was 6·5 mm.) so that hundredths could be estimated. Readings were made by means of a lens. These thermometers had been carefully compared with each other at temperatures ranging from 18° C. to 40° C., so that all readings could be corrected.

In order to determine the temperature within the limb, thermometers were inserted through slits in the skin between the muscles and allowed to remain there throughout the experiment. One thermometer ("distal") was inserted next to the inner surface of the gastrocnemius by passing it through the skin at one side of the tendon of Achilles and then back well up under the belly of the muscle. For the other thermometer ("proximal") an opening was made in

the skin opposite the popliteal fossa. Through this the thermometer was passed upward and underneath the thigh muscles. Both hind limbs were thus supplied with thermometers. The rectal thermometer was inserted as deeply as possible into the rectum. Although one limb served as a control, the temperatures in the limb to be denervated were determined over a period of at least one hour preceding the cutting of the nerves. The thermometers were not changed in position, once the experiment was started.

*Temperature increase in a denervated limb.*—The first experiment ran for ten hours following the denervation. The temperature of the denervated limb increased rapidly for ten minutes following the cutting of the nerves and then slowly during the remainder of one and one-half hours, after which they maintained a course parallel with those in the normal limb, but from 0·4° to 0·5° C. higher. The four thermometers in the limbs showed an increase in proportion to the increase in rectal temperature which developed during the experiment. The different temperatures at different stages of the experiment were as follows:—

| | Just Before Denervation. | 1·3 Hrs. after Denervation. | 2·5 Hrs. after Denervation. | 3·5 Hrs. after Denervation. | 9·7 Hrs. after Denervation. |
|---|---|---|---|---|---|
| Rectal..... .... ..... | 32·83°C. | 33·80°C. | 34·65°C. | 35·30°C. | 36·06°C. |
| Denervated "proximal" limb. | 31·80 | 33·40 | 34·00 | 34·60 | 35·25 |
| Normal "proximal"..... | 31·70 | 32·90 | 33·55 | 34·15 | 34·75 |
| Denervated "distal" limb... | 30·15 | 32·62 | 33·3 | 34·0 | 34·20 |
| Normal "distal" limb..... | 30·40 | 31·83 | 32·8 | 33·7 | 33·4 |

In a second experiment of this kind the thermometers had been sterilized and aseptic precautions were observed in inserting them beneath the muscles. The temperatures (Fig. 4) were taken at 10 minute intervals for 50 minutes preceding the sectioning of the nerves. Afterwards they were read at intervals of at first five minutes, then fifteen minutes, later thirty minutes, and finally one hour and two hours in length. The temperatures were registered for 60 hours after cutting the nerves. The animal appeared to be in good condition up to the forty-fourth hour, and remained under the influence of urethane. At that time a fever developed, due to tracheal infection. It died from occlusion of the trachea by mucous and pus at about the sixty-third hour. Throughout the experiment the temperature of the normal limb tended to keep parallel with the rectal temperature, but from 0·5° to 0·9° C. below. This difference became

greatest when the room temperature was lowest, especially in the region of the distal thermometer. On the other hand the temperature of the denervated limb increased 0·4° C. during the first ten minutes after cutting the nerves. This increase became gradually greater during the first hour until it was about 0·5° C. more than the normal limb.

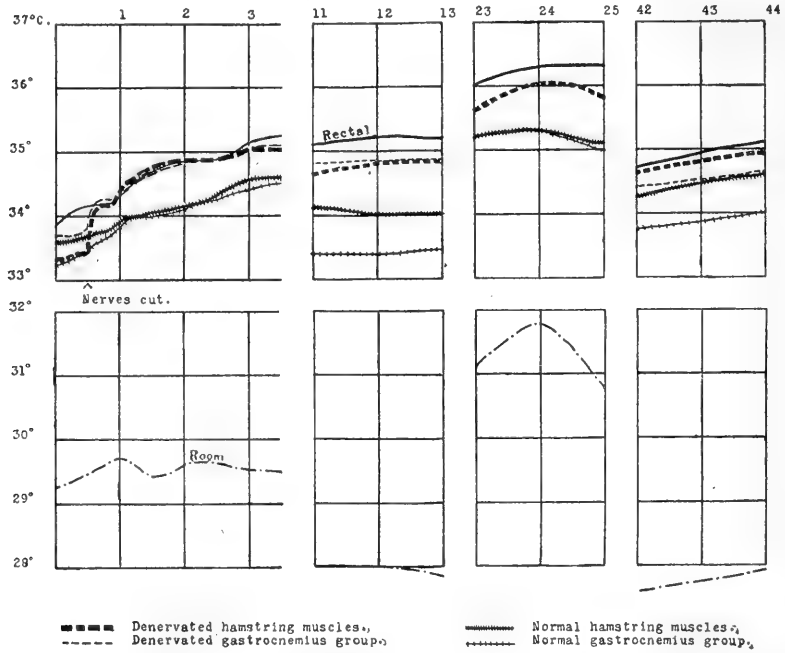


Fig. 4. A comparison of temperatures within the normal and denervated hind limbs of a cat. Shows the effect of varying the room temperature, upon "distal" and "proximal" temperatures in the normal and denervated limbs. Time, hours.

There was some fluctuation in the temperature difference between the normal and denervated limbs, it being greatest when the general body temperature (as determined by rectum) was lowered by allowing the room temperature to fall. Then the difference between the proximal thermometers became as great as 0·9° C. while that between the distal thermometers was as large as 1·5° C. The temperature difference became least when the general body temperature was greatly raised. Then the proximal and distal thermometers registered nearly the same, while the difference in temperature between the normal and denervated limbs became as small as 0·25° C.

In a third cat similar changes in temperature were produced (Fig. 5). There was a decrease of 0·45· in the normal limb, following denervation of the opposite limb, perhaps due to general vascular constriction, which takes place to counteract the dilator effects of denervation on general blood pressure. As before, the temperature of the denervated limb, although it soon exceeded the rectal temperature by about 0·2°, kept very near the rectal for several hours. Between the fifth and sixth hours the rectal temperature became less than that of the denervated limb. And in twenty-four hours the latter had decreased so much that the difference between the two was 0·7° to 0·8°. Changes in the room temperature influenced the temperature of the normal limb, as was to be expected, but had little or no effect upon that in the denervated limb.

*Temperature decrease in a denervated limb.*—Having determined the temperature increase, we next proceeded to find out the limits of temperature decrease in a denervated limb. This is of interest in connection with treatment, especially massage, of denervated muscle. The decrease in temperature may be due in part to constriction of the blood vessels. In such a condition massage might assist the circulation at least temporarily.

The time of "over-recovery" of a denervated limb is of considerable variation in different individuals. In order to discover when the "over-recovery" had taken place, the relative temperatures of the paws of the denervated and normal limbs was determined every few days by a method which Dale and Richards describe (7). The paw was immersed in a test tube containing 10 c.c. of water at room temperature. Movement of the paw stirred the water. The temperature of the water was read at one-minute intervals until five minutes had elapsed. This method, although not very accurate, indicated any decided differences between the two limbs. When we wished to obtain the limb temperatures more accurately at any time, the animal was anæsthetised with ether and "distal thermometers" inserted between the muscles as described above, aseptic precautions being observed. As soon as the temperature reading of the thermometer became constant, the thermometers were withdrawn and the skin brought together by sutures. This method, naturally, could not be used very often.

*Exp.* 1.—The femoral and sciatic nerves were cut in the right leg of a cat (1·5 kgm.). Five days later the temperature of the denervated paw was decidedly less than that of the normal. Seven days after denervation the animal was anæsthetised and thermometers inserted beneath the gastrocnemii. The temperatures were as follows: Rectal, 38·2° C.; normal limb, 36·7° C.; denervated limb

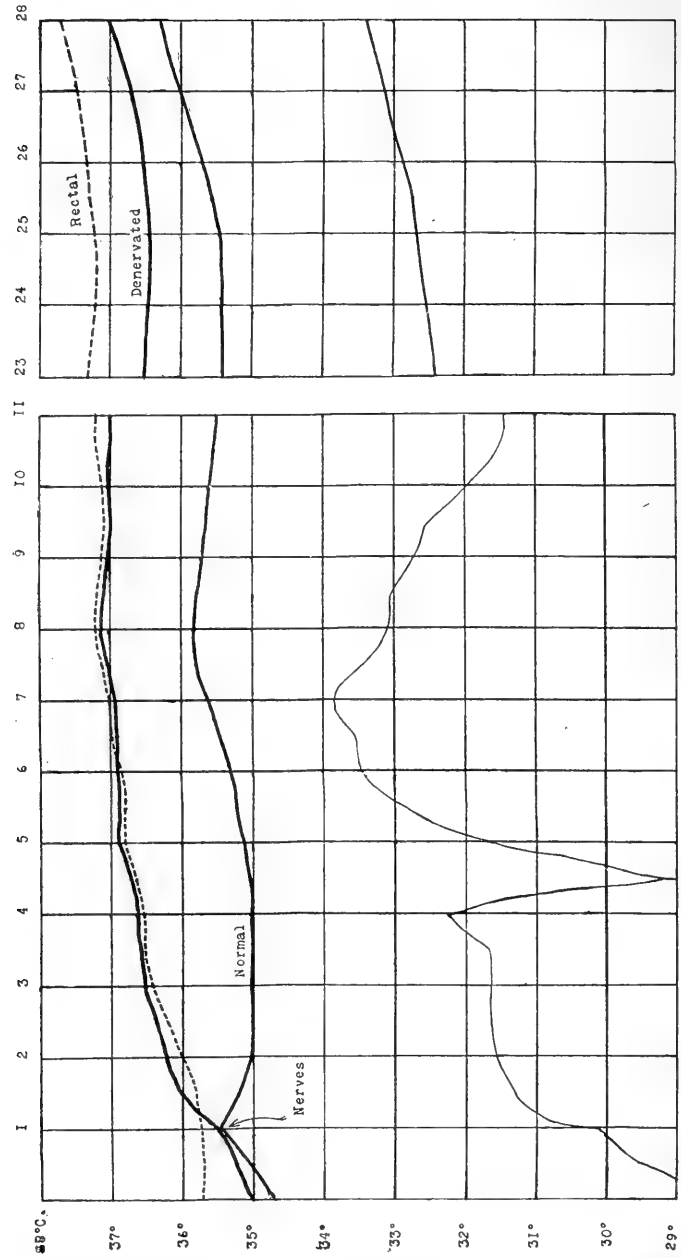


Fig. 5. A comparison of the temperatures of normal and denervated muscles in the hind legs of a cat. Shows partial recovery of the temperature in the denervated limb. Time in hours.

35·92° C. This was unusually rapid recovery, in fact it was "over-recovery," for the operated limb was practically 0·8° C. colder than the normal limb.

*Exp.* 2.—The right hind limb of a cat was denervated, causing an increased temperature which persisted for something more than two weeks. But in twenty-nine days there had been a decided "over-recovery." On the thirty-third day after the operation the denervated limb was 0·4° C. colder than the normal limb, as determined by thermometers inserted beneath the muscles.

*Exp.* 3.—In a third cat there was no decided "over-recovery" in eighty-six days. That is to say, the operated limb was slightly warmer (about 0·1° C.) than the control when the animal was deeply under ether, but if allowed to come out of the anæsthetic, the control limb became 0·3° C. warmer. The nerve had regenerated as far as the gastrocnemius (determined by electrical stimulation). There had been marked atrophy of the gastrocnemius. The denervated gastrocnemius-soleus-plantaris group weighed only 4·25 gm. as compared to 15·85 gm. the weight of the control group.

*Exp.* 4.—A fourth animal showed "over-recovery" of the denervated limb in fifty-six days. This condition persisted for weeks. As late as 151 days after denervation the temperature of the operated limb (thermometers under hamstring muscles) was 1·6° C. lower than the control. However the animal was in poor condition at that time. The nerve had regenerated to the gastrocnemius. It is interesting to note that the veins in the operated limb were much enlarged. The femoral vein was 7 mm. in diameter in the operated limb, while the diameter of the corresponding region of the femoral vein on the control side was 4 mm.

Fibrillation might be in part responsible for the increased temperature which often persists for weeks. Langley and Kato (5) have observed it from the fifth day to as far as 71 days after denervation. Their method was to study the exposed muscle with reflected light. The question occurred to us whether the fibrillation might be due to the stimulation resulting from exposure. We have made the following observations in this connection:

Rabbit. Sciatics cut 15 days before. Exposed gastrocnemius fibrillation immediately. This increased with exposure. Rubbing the surface of the muscle with absorbent cotton increased the fibrillation.

Rabbit. Sciatics cut 28 days before. Fibrillation was apparent in the gastrocnemius as soon as it was exposed. The temperature under this muscle was approximately the same as in the fore limb.

Rabbit. Both gastrocnemii had been denervated 42 days before. On exposure of left gastrocnemius, slight fibrillation was immediately evident, but this increased considerably during the first two minutes. Similarly the right gastrocnemius showed slight fibrillation immediately upon exposure, the fibrillation becoming more marked in the course of the folowing five minutes. The temperature of one of these limbs (thermometer inserted beneath gastrocnemius) was 34·78° C. as compared to 36·48° C., the temperature of a normal fore limb of the same animal. Fibrillation in the denervated muscle gradually disappeared after the animal was killed. It was slightly visible 40 minutes after the heart ceased to beat.

In all cases where fibrillation appeared it was visible immediately upon exposure of the muscle, but in many instances it was increased by the exposure. Moreover it has been shown by Schiff (8) that fibrillation occurs in unexposed muscle.

*Discussion.*

What is the cause of the increase in temperature in a denervated limb? Heidenhain (3) believed it was due to the increased circulation. This is supported by the observation that the increased volume of the denervated limb, as we have shown in § I, may persist for days, as does the temperature. The observation which questions this interpretation is the rapid recovery of the blood vessels after denervation, as determined by the venous blood flow (1). Recovery in such a case being only a matter of minutes.

As stated in § I, the temperature of the air within the plethysmograph was often maintained at the high level for hours after the limb had begun to decrease in volume. It is also difficult to reconcile this observation with the explanation that the temperature increase is entirely due to circulatory increase. It might be due in part to abnormal chemical changes. That abnormal changes are taking place is evidenced by the later appearance of fibrillation and the marked atrophy which occurs.

Every form of skeletal muscle contraction is no doubt accompanied by heat production. Therefore throughout the period of paralysis, beginning with the fourth or fifth day after denervation, a certain amount of heat must be produced attending fibrillation. However the quantity generated in this way may be quite small, for during later stages of paralysis the temperature of the muscle frequently becomes markedly lower than that of a normal muscle. Yet we have shown that fibrillation may still be present in such a case.

It is possible that the source of increased heat, which is present soon after denervation, is a chemical change independent of fibrill-

ation. It must be admitted that such an explanation is merely hypothetical, yet it would reconcile the disagreements of volume change and venous blood in relation to the temperature.

## Summary.


### *Volume changes.*

1. The maximum dilatation of the limb occurs from two to six hours after denervation. The extent of this dilatation is probably a little more than 2 p.c. of the total volume.

2. In some individuals, constriction of the denervated limb begins soon after the point of maximum dilatation has been reached. In others, constriction may not begin for a few hours after this time.

3. Complete recovery of the original volume occurs in many cases within twenty-four hours. There may be an "over-recovery" of the original volume as time goes on.

4. Constriction of the denervated limb may take place without a proportionate lowering of the limb temperature.

### *Temperature changes.*

1. The duration of the increased temperature resulting from denervation is exceedingly variable.

2. In many cases there is an "over-recovery" of the temperature. This occurs from a few days to several weeks after denervation.

3. Increased circulation and fibrillation do not seem to account entirely for the maintained supernormal temperature of a denervated limb.


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# STUDIES IN THE REGENERATION OF DENERVATED MAMMALIAN MUSCLE.

## II. EFFECT OF MASSAGE.

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In a recent paper Langley and Hashimoto (1) investigated the effects of massage and electrical treatment in preventing the atrophy of denervated muscle. The atrophy was determined by weighing the excised muscles at the conclusion of the experiment. This method cannot take into account the connective tissue present. In a denervated muscle this tissue becomes proportionately greater as the time is extended. On this account we have attempted to measure the actual power of a muscle to lift a load. This gives an idea of the amount of functioning muscle tissue present. In the present research we have confined ourselves to the study of the effect of massage.

*Methods.*—The muscles of the lower part of both hind limbs in the rabbit were denervated under aseptic conditions while the animal was under the influence of ether. This was accomplished by either cutting the sciatic nerve and then uniting the cut ends by means of catgut sutures or else by crushing the nerve against a glass rod with a stout thread according to Langley's method.

One of the most difficult problems in working with denervated limbs is their protection against abrasion. This is particularly so in the rabbit because it moves with a hop and when the sciatic is cut the toes are no longer able to take up the shock, which must therefore fall entirely upon the heel. Moreover denervated tissue is more easily injured than normal tissues, due in part, no doubt, to loss of the sensory information from those parts.

We first attempted to protect the heel by soft bandages, but two serious objections presented themselvs; some animals would succeed in loosening the bandages so that they slipped off, or at other times they might be wound so tightly that the foot became swollen. Leather

boots seemed to be an improvement in that they did not come off and did not interfere with the circulation. They were given a thorough trial on a number of animals. Their use had to be abandoned when it was found that many of the rabbits persisted in gnawing great holes in them and sometimes went so far as to eat away a third or a half of the boot. This together with the fact that a few animals persisted in gnawing away their toes suggested the idea of metal boots. After considerable experimentation the following boot was devised.

The boots were made from thin sheet aluminium (Figs. 1 and 2). Movement at the heel was permitted by a brass hinge. The boot was held on the animal by a wire which passed through slits at the back of the upper part and the top of the forward part (*a*, Figs.

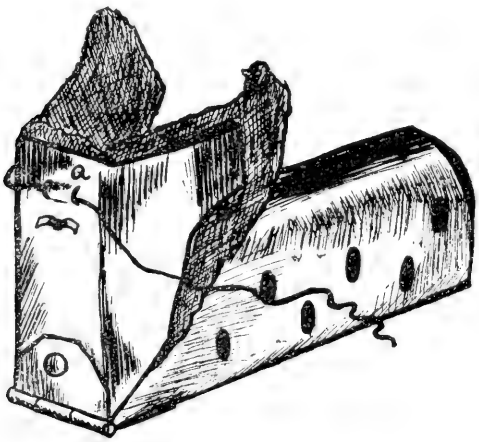


Fig. 1. Side view of boot.

1 and 2). The boot was fastened to conform to the resting position of the limb, *i.e.* with the foot flexed. The top piece and the forward part on the bottom and at the edge (*b*) where rubbing might occur were lined with flannel. Ventilation was afforded by holes in the sides and at the front. This was necessary in hot weather. As an additional safeguard absorbent cotton was placed under the heel and sometimes where the upper edge of the forward part of the boot (*b*) might rub. Wherever the hair was worn away or the skin became chafed, collodion was applied.

Massage was given by volunteer masseuses from the School of Massage, Hart House. In all cases the right leg was massaged by

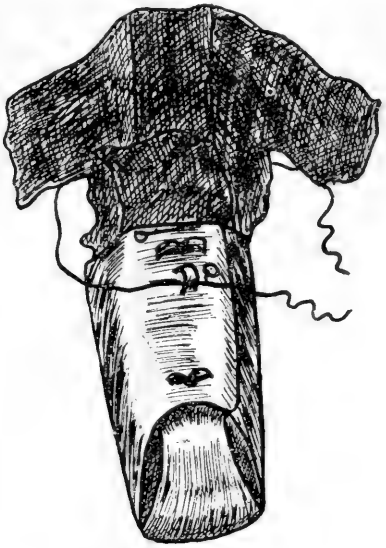

Fig. 2. Top view of boot.

the methods considered most appropriate for small muscles, viz., a gentle kneading and stroking. Both the left and right legs were put through passive movements three times to prevent stiffening of the joints. Such treatment was given from five to six days per week.

At the termination of the experiment, the rabbit was anæsthetised with urethane administered by a stomach tube; the muscles which have their insertion in the tendon of Achilles were carefully dissected out so as to disturb their circulation as little as possible; and then the animal was placed belly downwards on an animal board so that the hind limbs extended beyond the board. The tibia was held firmly by a clamp in such a way that the group of muscles (gastrocnemius, soleus and plantaris) to be tested hung freely as soon as the tendon of Achilles was cut. Drying of the muscle was prevented by frequent application of Ringer's solution or by smearing the outer surface with vaseline. The latter method seemed to give just as good results as the former.

The tendon was fastened to a lever from which at the same point was suspended a scale pan (Fig. 3). Both limbs were prepared at

the same time in the same way so that simultaneous records could be obtained of the corresponding groups of muscles on the treated and untreated side.

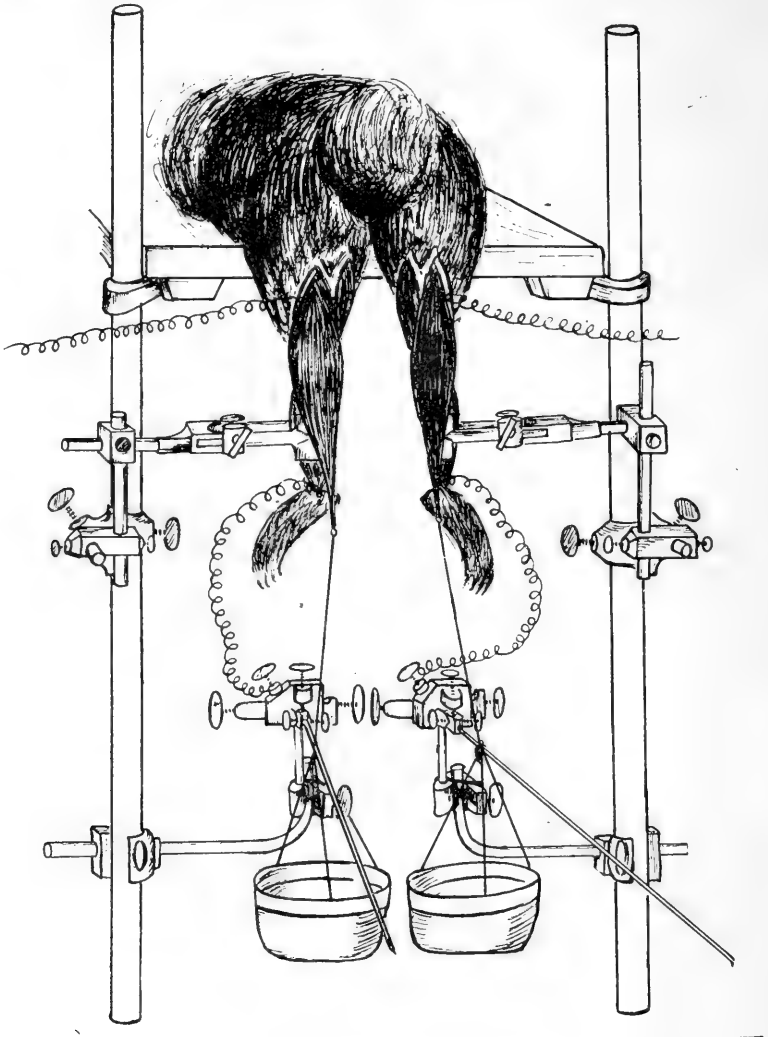

Fig. 3. Apparatus for testing muscles.

The muscle was stimulated by either induced or direct currents. In a majority of cases the induced current was used. When the former was used, one fine wire was fastened to the tendon and another above the origin of the muscles around bone and muscle. In the case of the direct current, cotton strips soaked in Ringer's solution connected the above described regions of the muscle with non-polarisable boot electrodes. The muscle was after-loaded. Care was taken to insure equal initial tension for the two muscle groups. The strength of current necessary to produce maximum contraction was employed. A series of contractions from gradually increased weights was obtained from each muscle group. In order to rule out variations resulting from circulatory changes due to ether anæsthesia, either simultaneous contractions of the two groups were secured or else the contraction of one group immediately followed the contraction of the other group for each increment. When the rabbits were placed under the influence of urethane the fluctuation due to anæsthesia did not occur.

Thus a series of contractions, with loads increasing by a definite increment, as 2 gm., 4 gm., or 10 gm., depending upon the power of the muscle, was obtained for the treated and untreated denervated muscles under similar conditions. The load often went as high as the absolute limit. In order to see whether fatigue in any way altered the relative power between the muscles of the two sides as many as six sets of records were made in many tests. From these a typical set was chosen for plotting the work curve.

As soon as the functional tests of the muscles were completed, the muscles on each side were carefully removed and weighed with the observation of such precautions as similar dissection and prevention of drying (*see* Langley (1), p. 16). The muscles were then fixed in Orth's solution for histological study.

Load and work curves were plotted from all records obtained. The curve for the treated muscle group was drawn on the same sheet with that for the untreated muscle group. Thus corresponding parts of the curves could be compared at a glance.

*Results.*—An indication of the variation between the right and left gastrocnemius-soleus-plantaris group was obtained from a study of fifteen normal rabbits (Table I). A comparison of the work performed at the optimum load gives a fair idea of the two curves for comparison. It is to be noted, too, that the heavier muscles do not always have the greater capacity for work, although there is a surprising tendency in that direction. Moreover the left muscles were stronger than the right in nine of the fifteen animals. Treated animals in which sores developed were discarded if the muscles were affected in any way.

The percentage difference between the corresponding muscle groups varied so greatly that to strike an average would mean little. In view of this and not knowing what the muscle could do before denervation it would be necessary to obtain a very marked increase of power in the treated muscle as compared to the control to prove that massage was of value.

The duration of the massage was increased from week to week in many cases, as the table indicates. It being assumed that the massage might be needed more as the circulation decreased. The principal indication that the circulation was decreased, being the lowered temperature in long-standing denervations.

Thirty-seven animals were successfully carried through to completion. The duration of the treatments ranged from seven to 190 days. Twenty-three of these possessed stronger muscles on the treated side, the remaining fourten having stronger muscles on the untreated side. In those animals in which the treated muscles were stronger they average 64·2 p.c. greater than the control muscles, while in those rabbits possessing more powerful control muscles, the average increase over the treated muscles was 43·7 p.c.

TABLE I. A comparison of weights and work performed at the optimum load in normal gastrocnemius-soleus-plantaris muscle groups.

| Animal. | Work at optimum load in gm.-mm. | | Percentage difference, right referred to left. | Weight in grams. | | Percentage difference. |
|---|---|---|---|---|---|---|
| | Right. | Left. | | Right group. | Left group. | |
| 38 | 82 | 79 | – 2 | | | |
| 39 | 128 | 202 | – 37 | 8·46 | 8·86 | – 4·5 |
| 40 | 72 | 117 | – 38 | 9·16 | 9·61 | – 4·7 |
| 41 | 65 | 67 | – 2·9 | 11·61 | 12·06 | – 3·7 |
| 42 | 158 | 203 | – 22 | 9·46 | 9·66 | – 2·07 |
| 43 | 102 | 147 | – 30·6 | 9·7 | 9·8 | – 1·04 |
| 44 | 263 | 240 | 9·6 | 9·30 | 9·01 | 3·2 |
| 45 | 60 | 78 | – 23 | 11·58 | 11·66 | – 6·85 |
| 46 | 79 | 65 | 21·6 | 10·23 | 10.45 | – 2·1 |
| 47 | 65 | 179 | – 63·6 | 16·10 | 15·76 | 2·16 |
| 48 | 77 | 64 | 20 | 12·31 | 11·83 | 4·05 |
| 49 | 84 | 157 | – 46·5 | 14·70 | 15·06 | – 2·4 |
| 50 | 88 | 73 | 20 | 14·05 | 14·13 | – 5·66 |
| 51 | 63 | 59 | 6·7 | 13·60 | 13·91 | – 2·2 |
| 52 | 194 | 156 | 24 | 17·26 | 17·03 | 13 5 |

The normal muscles of Table I showed a predominance of left over right, not only in the number of animals involved, but in the percentage of differences. Where the left muscle group was greater in power, the average was 29·5 p.c. over the right, while in the cases of right preponderance the muscles averaged only 15·9 p.c. more power-

ful than the left. If it should turn out to be true that rabbits, on the average, possess more powerful muscles in the left leg than in the right, that would strengthen the observations in Table II and indi-

TABLE II. Comparison of treated and untreated denervated muscle.

| Animals. | Duration of massage each day in min.* | Duration of treatment in days. | Work at optimum load in gm.-mm. | | | Weight in grams. | | |
|---|---|---|---|---|---|---|---|---|
| | | | Right group (massaged.) | Left group (control.) | Percentage difference, right referred to left. | Right group | Left group. | Percentage difference, right referred to left. |
| 1 | 3 | 7 | 59 | 29 | 104 | 9·03 | 9·08 | 0 |
| 2 | 3 | 11 | 66 | 43 | 53 | 9·63 | 9·65 | 0 |
| 3 | 2 | 13 | 24 | 17 | 41 | 7·81 | 7·81 | 0 |
| 4 | 3, 4 | 14 | 111 | 292 | − 62 | 7·63 | 8·25 | − 8 |
| 5 | 30 | 15 | 130 | 152 | − 15 | 6·40 | 7·79 | −18 |
| 6 | 3, 4 | 17 | 260 | 188 | 37 | 7·38 | 7·26 | 2 |
| 7 | 3, 4 | 20 | 287 | 112 | 156 | 9·26 | 8·80 | 5 |
| 8 | 30 | 20 | 272 | 334 | − 19 | 5·3 | 5·2 | 2 |
| 9 | 3, 4 | 25 | 147 | 135 | 9 | 7·11 | 7·91 | −11 |
| 10 | 3, 4, 5, 6 | 26 | 137 | 54 | 154 | ..... | ..... | ..... |
| 11 | 3, 4 | 29 | 290 | 181 | 60 | 7·67 | 7·11 | ..... |
| 12 | 3, 4, 5, 6, 7 | 30 | 187 | 129 | 45 | 6·29 | 5·86 | 7 |
| 13 | 3, 4, 5, 6 | 34 | 133 | 189 | − 42 | 5·06 | 5·48 | − 8 |
| 14 | 3, 4 | 36 | 104 | 224 | − 54 | 4·7 | 4 65 | 2 |
| 15 | 3, 4 | 38 | 54 | 47 | 15 | 7·51 | 8·18 | − 9 |
| 16 | 3, 4 | 40 | 57 | 98 | − 45 | 6·43 | 6·91 | − 8 |
| 17 | 2, | 41 | 87 | 59 | 47 | 4·85 | 4·80 | 0 |
| 18 | 3, 4 | 44 | 273 | 190 | 44 | 6·2 | 5·7 | 9 |
| 19 | 2, 3, 4, 5 | 44 | 190 | 263 | − 28 | 4·10 | 4·12 | 0 |
| 20 | 2, 3, 4, 5, 6 | 45 | 184 | 128 | 44 | 6·4 | 5·45 | 18 |
| 21 | 3, 4 | 45 | 158 | 251 | − 37 | 6·8 | 6·6 | 3 |
| 22 | 2, 3, 4 | 48 | 40 | 13 | 208 | 4·23 | 3·94 | 8 |
| 23 | 2, 3 | 49 | 38 | 28 | 36 | 5·1 | 4·2 | 21 |
| †24 | 3, 4, 5, 6, 7 | 50 | 37 | 30 | 26 | ..... | ..... | ..... |
| ‡25 | 3, 4, 5, 6, 7, 8, 9, 10 | 61 | 140 | 52 | 169 | 7·86 | 7·82 | 1·4 |
| ‡26 | 3, 4, 5, 6, 7, 8 | 65 | 141 | 107 | 32 | 7·30 | 7·87 | − 8 |
| 27 | 3, 4, 5, 6, 7, 8 | 68 | 80 | 83 | − 4 | 7·56 | 8·71 | 15 |
| 28 | 3, 4, 5, 6, 7, 8, 9, 10 | 78 | 38 | 55 | − 44 | 5·44 | 5·30 | 3 |
| ‡29 | 3, 4, 5, 6, 7, 8, 9, 10 | 81 | 34 | 71 | −109 | 5·80 | 5·10 | 14 |
| §30 | 3, 4, 5, 6, 7, 8, 9 | 83 | 111 | 49 | 123 | 6·64 | 6·87 | − 3 |
| 31 | 2, 3, 4 | 91 | 258 | 250 | 34 | 6 5 | 6·5 | 0 |
| ‡32 | 3, 4, 5, 6, 7, 8, 9, 10 | 93 | 123 | 287 | −133 | 5·79 | 7·41 | −28 |
| ‡33 | 3, 4, 5, 6, 7, 8, 9, 10 | 164 | 98 | 76 | 29 | 9 55 | 8·56 | 11 |
| 34 | 3, 4, 5 | 105 | 136 | 100 | 36 | 8·32 | 8·48 | − 2 |
| 35 | 3, 4, 5, 6, 7, 8, 9 | 152 | 157 | 183 | − 14 | 11·5 | 12·5 | − 8 |
| ‡36 | 3, 4, 5, 6, 7, 8, 9 | 190 | 234 | 216 | 8 | 12·50 | 12·20 | 2 |
| ‡37 | 3, 4, 5, 6, 7, 8, 9 | 190 | 344 | 361 | − 6 | 14·6 | 15·4 | 7 |

* Where a series of numbers is given, it means the number of minutes each succeeding week, the treatment being maintained at the last figure for the balance of the time.

† Treated 30 days, then without treatment for 20 days before testing.

‡ Nerve regenerated as determined by stimulation or by voluntary movement of toes.

§ Conduction in right tibial nerve, but not in left.

cate some benefit from massage. However the number of animals used in Table I was too small to establish such a generalisation.

A very important consideration must be kept in mind, and that is concerning the atrophy of denervated muscle. Such a muscle dwindles in volume and no doubt in power, so that if the massaged muscle is compared with the corresponding untreated muscle by its capacity to do work, an increase of 64 p.c. over the control does not mean so much as it would in normal muscles. In other words both treated and untreated muscles dwindle rapidly, but the massaged muscle maintained 64 p.c. more strength. The treated muscles might lose a great deal and still do that. Even this might be of more significance if a large majority of the animals treated showed this, but this is true in only twenty-three out of thirty-seven animals treated. Slightly more than a third of the number showed an opposite condition.

Judged from the functional test, therefore, massage has not proven to be of great value in our experiments. But because we did not know the relative capacities of the muscles before denervation, we cannot make our conclusions as positive as might be done otherwise. Moreover the method of testing is open to the objection that there is some disturbance of the circulation in the preparation of the muscle so that its tendon can be fastened to the lever. Although the two sets of muscles were prepared in as similar a manner and as quickly as possible, a certain amount of disturbance in the muscle was unavoidable.

There are certain observations which we will point out in reference to the comparative weights of treated and untreated muscles. In the first place the percentage difference in weight never reaches the range attained in the functional test. In the second place if the functional test shows a much greater preponderance of one muscle group over the other that muscle group frequently weighs less than the other. All told, out of the thirty-seven experiments, seventeen did not agree in functional and weight tests as to which preponderated.

These observations seem to indicate that the weight of a muscle does not necessarily indicate the relative amount of contractile tissue.

In conclusion we wish to thank Miss Joan Campbell, who gave valuable assistance in this research. We wish also to thank the members of the Schol of Massage at Hart House for their part in the research.

## Summary.

1. The soleus, gastrocnemius and plantaris muscles were denervated on both sides in thirty-seven rabbits. The muscles of the right side were massaged from two to ten minutes a day over periods varying from seven to 190 days. At the conclusion of the experiment the work capacity of the treated muscles was compared with that of the control. The two groups of muscles were also weighed.

2. In a similar manner the same muscles in fifteen normal animals were compared on both sides.

3. Sixty per cent of the normal rabbits possessed stronger muscles on the left side. In the cases of left preponderance the difference was much greater than in the cases of right preponderance. A large proportion of the left muscle groups were also heavier than the right, although such muscles did not invariably prove to be the stronger.

4. The massaged muscles were stronger than the controls in sixty-two per cent of the animals treated. There was considerable discrepancy between the comparison by weight and the comparison by function.

5. In view of our ignorance of the relative capacities of the two sets of muscles before beginning treatment, a small predominance of power in the treated muscles is inconclusive. Our observations indicate that massage of denervated muscle is slightly beneficial[1].

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[1] A subsequent study upon the effect of massage on denervated muscle, involving a larger number of animals and a method of testing the muscle functionally both before operation and during the period of treatment will appear later.

# UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES


No. 31: THE FUNCTIONAL PATHOLOGY OF SURGICAL SHOCKS, BY J. J. R. MACLEOD.

(REPRINTED FROM THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, VOL. V)

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# THE FUNCTIONAL PATHOLOGY OF SURGICAL SHOCK

BY

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## *The Functional Pathology of Surgical Shock*

BY CONCERTED investigation in experimental medicine in England and in this country, remarkable progress was made during the last two years of the war in the elucidation of the causes of the condition known as shock. There are several varieties of this condition, the two most characteristic of which are surgical shock met with in the operating room and after severe accidents in civic practice, and secondary trench shock met with at the battle front. In every essential particular these two conditions appear to be alike, and a condition apparently identical with them can be produced in laboratory animals by various experimental procedures. It is largely because of the availability of this experimental material that it has been possible to throw so much light on the problem. This but serves once again to illustrate the necessity of animal experimentation in the furtherance of medical and surgical knowledge. Had it not been for the work done on shock in the laboratory by Bayliss, Cannon, Dale, Erlenger, and others, the war might have ended without our being any further advanced in our knowledge of this mysterious and fatal condition. It may be of interest here to review very briefly some of this experimental work.

We shall consider first of all the investigations by Dale,[1] and Laidlaw and Richards[2] on the shock-like condition which is produced by injections of histamine (iminazylethylamine). This substance is derived by removal of the carboxyl group, as $CO_2$, from histidine, one of the most important of the building stones of the protein molecule. Injected quickly into etherized animals in very minute dosage (1 mg. per kg. body weight) histamine soon causes the arterial blood pressure to fall to the shock level of 30-40 mm. Hg. For a brief period preceding the fall there is a rise in pressure due to constriction of the arterioles, and this constriction persists while the pressure is falling. So far as the obvious vascular changes are concerned, therefore, the condition is strictly comparable with those found in shock—low blood pressure and constricted arterioles. By the time the pressure has fallen to near the shock level the cardiac pulsations disappear from the tracing. The respirations also cease, but if the animal be kept alive by artificial respiration and the thorax opened for inspection of the heart this organ will be observed to be beating quite vigorously, with, however, a pronounced deficiency of blood in the auricles and in the large veins both of the thorax and abdomen. This observation affords positive proof that in this form of shock at least the fundamental cause for the condition is inadequate blood flow to the heart. The question is, what becomes of the blood? Either it must pass out of the blood vessels into the tissues, or the capacity of the former must be increased. Loss of blood itself could scarcely occur short of hemorrhage—of which there is no evidence in histamine shock—but the water with some of the soluble constituents (plasma) might become extravasated, leaving in the vessels blood excessively rich in corpuscles. Such extravasation actually occurs in acute histamine shock, as revealed by measurement either of the concentration of hem-

oglobin or of the corpuscles, but this in itself can not explain all of the loss in circulating blood, for if the histamine be given slowly (over a period of 20-30 min.) it takes much longer for the shock to become established, and the blood does not show any increase in the percentage of hemoglobin or in the number of corpuscles. In these cases we are driven to conclude that much of the blood must be withdrawn from currency by stagnation in dilated vessels. Direct evidence for this important conclusion has been secured by determination of the volume of circulating blood, by means of the vital red method of Keith, Rowntree and Geraghty,[3] described elsewhere.

Although the oligemia is due in great part to dilatation of the capillaries and venules of the intestine, as can be shown by inspection, it is also partly dependent upon dilatation of vessels elsewhere, since histamine shock can be induced in animals from which all of the intestines have been removed. The vessels of the skeletal muscles are probably the chief extraabdominal vessels affected, for although no dilatation of these can ordinarily be seen in histamine shock, it becomes quite evident in animals which have been transfused before being shocked. The capillaries (and venules) in these areas evidently lose their tone so that they become too roomy for the available blood. As a matter of fact Dale and Richards[2] have shown that histamine abolishes the tone of capillaries at the same time that it increases the permeability of the walls and so permits the plasma to leak through. It is on account of this latter action that histamine when it is rubbed on the scarified skin soon causes the formation of a wheal like that following the lash of a whip.[4]

When histamine is given to unanesthetized animals about ten times as much can be withstood as in those that are anesthetized with ether.[5] At first sight this result might seem to discount the observations on etherized animals, but on the contrary they greatly enhance their importance. They indicate that whereas the normal animal is able to combat the toxic action of histamine, ether greatly depresses this power, an observation which agrees remarkably with the clinical experience that administration of ether is most dangerous in persons who are threatened with shock. The poisoning effect of ether persists for some time after the anesthetic is removed, and it is no doubt dependent upon a toxic action on the endothelium of the capillaries, for it is particularly in such animals that concentration of the blood is evident after histamine. It is of great significance that histamine did not readily produce shock in nitrous oxide anesthesia.

Hemorrhage also greatly predisposes to histamine shock, but in this case the blood is not nearly so concentrated as ordinarily because of the passage of plasma from the tissue spaces into the vessels, which, it will be remembered, is the natural reaction of an animal to hemorrhage alone. The cause of shock in such animals is mainly the opening up of the vessels.

Many bacterial toxins, both when applied to scarified skin and when injected intravenously, have effects very like those of histamine. It is also well known that shock is peculiarly common after injuries in which there has been extensive destruction of tissue. The facts warrant the suggestion that shock may be due to liberation from damaged tissues, particularly the muscles and the viscera of toxic substances acting like histamine. This conforms with the fact that shock is most common when there has been extensive destruction of muscle, or when

the liver or intestines are roughly handled. It is possible also that the shock of intestinal obstruction is fundamentally due to absorption into the blood of similar substances from the closed loop of intestine. Whipple and Hooper's discoveries that absorption of a proteose is responsible for the shock-like symptoms of intestinal obstruction are very suggestive in this connection.[6]

But to return to surgical shock. Is it possible that the condition is dependent upon intoxication by histamine-like substances absorbed from greatly damaged tissues? To test this hypothesis Cannon[7] and others have investigated the effects of crushing the muscles of the hind limbs, without external hemorrhage, by blows from a heavy hammer. It was found that an immediate fall in blood pressure occurred, followed by a more gradual decline to the shock level, with a decrease in the $CO_2$-combining power and a marked concentration of the blood. This result was not due to irritation of afferent nerves, causing excessive stimulation of the vasomotor centers, since it persisted in animals in which all nerves of the limb had been cut; neither was it caused by any local loss of circulating fluid (by dilatation of vessels or extravasation). It was due to the discharge into the circulation of some toxic material, since no shock resulted when the vessels of the damaged limb were clamped. Removal of the clamp some time after the damage resulted in the immediate appearance of the symptoms which could again be caused to disappear somewhat by its reapplication. As to the nature of the toxic material, the first possibility to be considered is that it is unoxidized acid (lactic), which, it is well known accumulates quickly in muscular tissue whenever this is destroyed, or when the circulation through the tissues is greatly curtailed. As a matter of fact it was found that the $CO_2$-carrying power of the blood became greatly depressed whenever the toxic material was permitted to enter the circulation by removal of the clamp, and it is well known that there is also a decided depression in the blood carbonates in surgical shock. Acid intoxication can not, however, be the main factor, and for the following reasons: 1. Injections of lactic acid intravenously do not cause shock, neither do they predispose an animal to it. 2. Copious injections of bicarbonate solution do not prevent shock. 3. Extracts of damaged muscle made with isotonic saline do have a shock-like effect, but this is just as great when the lactic acid in the extracts is neutralized with bicarbonate, as when they are unneutralized. Moreover the fall in the blood carbonate does not coincide with, but rather precedes, the development of the shock symptoms. An excess of lactic acid in the blood has been noted in the later stages of many cases of shock (Wiggers and Macleod), but this is a secondary effect, and it is doubtful whether it is the only cause for the depressed $CO_2$-carrying power of the blood.

In one or two cases the muscles were crushed in unanesthetized cats, with the result that shock did not invariably follow, but this does not invalidate the observations on anesthetized animals; it only shows that, as in histamine poisoning, the anesthetic weakens the resistance. When the normal animals were bled before the crushing operation, shock supervened with certainty.

Taking the results as a whole and comparing them with clinical experience a very strong case is made for the hypothesis that surgical shock is essentially due to intoxication by materials derived from damaged tissue. Shock is particularly common after severe tissue damage; rough handling of the wound greatly

aggravates it, whereas rigid care to render the wounded part immobile is a valuable safeguard; the administration of ordinary anesthesia, (ether) to a shock patient is notoriously dangerous, whereas rapid amputation under nitrous oxide often ushers in a steady recovery. All these clinical facts conform admirably with the experimental findings.

With regard to the diagnostic value of measurement of the blood volume, it has been shown by Erlanger, Gasser and Meek[8] that concentration of the blood becomes evident before the shock symptoms are pronounced. This concentration is no doubt a most important factor in causing curtailment of the volume of circulating fluid, not only because of loss of plasma, but also because it causes the corpuscles to become contiguous so that they have a tendency to jam in the capillaries and so lead to a progressively increasing under-nutrition of the tissues and the production of more toxic material.

It remains for us to show that the foregoing conclusions drawn from observations made on laboratory animals are applicable to the clinical condition known as surgical shock. It will then be advantageous to consider the principles which determine successful treatment. The unusual opportunity afforded at the front to study shock has led to a furtherance of our knowledge of its causes, which might have taken many years of investigation in time of peace, and by far the most important contributions have come from those who have been intimately familiar with the experimental as well as the clinical aspect of the problem. N. M. Keith[9] estimated the total volume of circulating blood by the vital red method and the relative amounts of plasma and corpuscles by measurement of hemoglobin or by means of the hematocrit, and as a result of his investigations has divided the cases of secondary shock into three groups which vary from one another with regard to: 1. The total volume of blood in circulation and (2) the relative amounts of plasma and corpuscles in the blood. The differentiation is not only of great prognostic value, but also invaluable as a guide to the proper plan of treatment. In group 1 are the *compensated cases,* in which the blood volume is reduced to not more than 80 per cent of the normal, but in which the plasma is relatively greater, being reduced only to 85 or 90 per cent of the normal. In other words these cases have reacted like cases of hemorrhage, i. e., there has been a migration of fluid from the tissues into the blood. If kept warm and given fluid per rectum, the patients recover. In the second group, called *partially compensated,* the blood volume is reduced to 65-75 per cent, with little, if any, evidence of dilution of plasma (i. e., the plasma is also reduced to 65-75 per cent). Treatment by transfusion either with blood (citrated blood by Robertson's method,[10] or with gum solutions (*vide infra*) is necessary and in most cases, if the proper technic is followed in the transfusion, recovery is likely. It is important, however, that the plasma volume be measured a few hours after the transfusion to see whether the desired reaction, namely, a migration of fluid into the plasma, has set in. If not so, a second transfusion is indicated. In favorable cases the plasma volume increases more rapidly than that of total blood, and *pari passu* the arterial blood pressure rises.

In the third or *uncompensated group*—the blood volume is below 65 per cent and the blood is more concentrated than normal, i. e., there is relatively a greater decrease of plasma. Treatment must be energetic in these cases, but the

prognosis is unfavorable because the transfused fluid readily leaves the vessels, causing the lungs and tissues to become edematous.

With regard to the rationale of the transfusions, it is clear that the added fluid makes good the blood that is lost by stagnation, etc., and so tends to maintain in the circulation a normal pressure for a sufficient time to enable the organism to destroy the toxic bodies. If the shock condition has existed for some time, so that the nerve centers are paralyzed, the injections are of no avail. Since many cases of shock in man have also suffered considerably from loss of blood, it is often difficult to decide whether shock really exists apart from the effects of hemorrhage, the cardinal symptoms of the two conditions being very much alike. The test is afforded by examination of the total blood and plasma volume, and by the reaction to transfusion. After hemorrhage alone there is great migration of plasma into the blood, making this very dilute, and transfusion has immediately beneficial results. In shock there is no migration of fluid into the blood, indeed the reverse is usually the case, and transfusion does not always succeed in re-establishing normal conditions.

Finally, with regard to the composition of the transfusion fluid, should this be human blood, or can a reliable substitute be found in saline solutions containing gum? There is much diversity of opinion over this question. Keith sums up by stating that there does not appear to be any decided advantage in blood over gum solutions, although the immediate restoration of natural color to the patient, which occurs with blood but not with gum solutions, may make the former appear to be the more satisfactory treatment.

Much painstaking work has been done by Erlanger and Gasser[8] to determine the exact conditions for success in using gum solutions. As their criterion for successful treatment, they did not merely see whether the blood pressure was restored, but they allowed the animals to recover from the effects of the anesthetic and then watched them to see whether they became restored to normal. Many animals might appear to be recovering, but nevertheless succumb within 24 hours. These workers point out that strong gum solutions owe their efficacy to the fact that they slowly attract water into the blood from the tissues, and once attracted the water remains in the vessels. Hypertonic solutions of crystalloids on the other hand, quickly attract water, but this is not retained long. These workers, therefore, devised the scheme of combining the two factors, and they found that success depended on how this was attempted. In the shock produced by partial clamping of the vena cava about one-half of the animals died within 48 hours. Neither weak gum (6 per cent) and weak alkali (2 per cent) given in large amount (12 c.c. per kg.) nor strong gum (25 per cent) in strong alkali (5 per cent) given in smaller dosage (5 c.c. per kg.) decreased the above mortality; but if strong gum (25 per cent) were given along with strong glucose solutions (18 per cent) at the rate of 5 c.c. per kg. an hour, many more animals survived. The alkali was chosen to furnish the crystalloid, in many of the experiments, so that it might incidentally combat any existing acidosis. We have already seen, however, that there is no reason to believe that acidosis is an important factor in shock. Two precautions are necessary to success in using the gum solutions, first they must be properly prepared, and second they must not be injected so rapidly

that their high viscidity would slow the circulation and so embarrass the heart's action.

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—*J. J. R. M.*

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## PHYSIOLOGICAL SERIES

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# On Ventilation*

By J. J. R. MacLeod, M.B., Ch.B. (Aberdeen), D.P.H., (Camb.).
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INVESTIGATION of the functions of animals is essentially a more difficult problem than that of the physicist or chemist because variable and unknown factors dependent on the life process are involved. The reactions of an animal to changes in the environment are therefore not always strictly predictable, even when we consider only measurable objective phenomena. Many of the problems of physiology consist in a study of the relationship between conditions of the environment and the behaviour of isolated living tissues. These studies are entirely objective in nature. When similar studies are made on the animal as a whole, subjective phenomena also have to be considered, and the results are much less predictable.

The well-being of a conscious animal in relationship to its environment constitutes the main problem of the study of ventilation. In the case of animals living an outdoor life, it is a problem of relatively little importance, but for those like man which spend much of their time in confined spaces, it is a problem of great importance, for in them it becomes necessary to determine the limits within which the outside influences may be altered without detriment to health or comfort. It is the problem which can be solved only by an understanding of the principles of ventilation, and I propose in this lecture to indicate briefly the state of our present-day knowledge with regard to principles, and to show how this knowledge can guide us in the selection of means to improve the conditions in our living rooms and indoor public places.

It is often imagined that ventilation is a matter merely of pure air and that it therefore becomes a problem requiring attention

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only in cases where the air has been polluted by the crowding together of many people. It is considered the problem of the ventilation engineer alone and not one that applies in living rooms in which there is no overcrowding. I shall endeavour to show, however, that this attitude is a wrong one, and that there is very good evidence for the belief that much discomfort and ill health could be avoided if people understood more clearly the physical conditions of the atmosphere which bear a relationship to the well-being of the body.

When our knowledge of the function of breathing became developed to the extent of showing that an animal requires the oxygen of the air for the living processes of its body, and as a result of these processes that it produces carbonic acid, which is then added to the air, it was natural to suppose that the unfavourable effect of overcrowded confined spaces was due either to the using up of the available oxygen or to a poisonous action of the carbonic acid. Indeed, it is still the notion of many people that one or other of these changes in the air is the cause of the discomfort of living in crowded places. It is true that a great deficiency of oxygen, such as occurs sometimes in mines because of oxidative processes in the soil, will very quickly cause serious symptoms, often indeed will produce a suddenly fatal result. And a lesser deficiency such as occurs in those unaccustomed to the rarefied air of high mountains, or in aeronautics, is undoubtedly responsible for most of the untoward symptoms classified under the term of mountain sickness. But that $O_2$ deficiency is not a usual factor in the evil effects of vitiated atmospheres is made plain when we state that even in the most overcrowded room a decrease of $O_2$ of one per cent.—*e.g.*, from 21 to 20—is practically never overstepped, a decrease which, however, is very much less than that which occurs at altitudes in which, after acclimatization, people live in perfect condition. Many of our best known health resorts and sanataria indeed are situated at altitudes in which the percentage of oxygen is greatly reduced, and there are large cities situated at altitudes of 8 or 10 thousand feet. In Pitosi, a city on the high plateaux of the Andes, the percentage of oxygen calculated at sea level is not more than 12 per cent., a reduction of 9 per cent. below the normal, and yet the people of this city are well and healthy and capable of as much effort as those living at lower altitudes. "Girls dance half the night and toreadors display their skill in the bull-ring."

Clearly, therefore, oxygen deficiency has nothing to do with the evil effects of ill-ventilated places.

With regard to a deleterious effect of the accumulated $CO_2$, similarly negative results have been obtained. This bold statement may possibly surprise some of you, for no doubt you have known that for a great many years the contrary was believed and that the percentage of $CO_2$ in the air was taken as the criterion of the adequacy of ventilation. So firmly rooted indeed has this conception become that it has required considerable investigation to overthrow it. Although it has been known to physiologists and hygienists for many years that accumulation of $CO_2$ has nothing to do with the evil effects of polluted air, it is still believed by the laity to be the really important factor, so slowly does the work of the scientist find its application in the life of the community.

It is not altogether easy to understand why excess of $CO_2$ was thought to be the important factor responsible for the evil effects of vitiated atmospheres. No doubt the chief reason was that the percentage of this gas is often raised in such atmospheres, but this is nothing more than coincidence, for, on the one hand, most unsuitable conditions may exist when the percentage of $CO_2$ is normal, and on the other, air loaded with almost a hundred times the percentage found even in the most polluted atmosphere can be breathed for indefinite periods of time without any unfavourable symptoms.

As a matter of fact, even in the open, we are constantly taking into the air sacs of the lungs large percentages of $CO_2$, for obviously with each inspiration the first air to be drawn in is that which remains over in the air passage from the preceding expiration. This air contains somewhere about 5 per cent. of $CO_2$, and in quiet breathing it amounts in volume to about one-third of all the air that is drawn in from the outside. This alone indicates that $CO_2$ *per se* cannot be poisonous, and when we consider further the now well-known fact that a certain amount of this gas in the air sacs is absolutely essential to the well-being of the animal, the whole hypothesis of its toxic action becomes, to say the least of it, absurd. Indeed, so important is the presence of this constant amount of $CO_2$ in the alveolar air that whenever there comes to be a marked increase in the amount of $CO_2$ in the atmosphere, the breathing becomes greater, so as to ventilate the air sacs more thoroughly, and thus keep the relative amount of $CO_2$ in them at the normal level. The extent of this increase in respiration is usually so small as to be unnoticed by the individual, and certainly increased breathing is not one of the symptoms of which persons complain who are living in polluted atmospheres. Furthermore,

not only man, but other animals as well, frequently breathe by choice under conditions which cause great increase in the $CO_2$ content of the inspired air. "Not only the new-born babe sleeping against its mother's breast, but pigs in a stye, young rabbits, rats and mice clustered together in their nests, young chicks under the brooding hen, all alike may breathe a higher percentage than that legally allowed in spinning mills or weaving sheds." . . . "In breweries the men who tend the fermentation vats work for long hours in concentrations of $CO_2$ of 0.5—1 per cent. Such men are no less healthy and long lived than those engaged in other processes of the brewery trade." (Leonard Hill.)

In face of such evidence, even the most ardent supporters of the theory that the vitiated air owes its evil influence to $CO_2$, were compelled to abandon their position, but they did not do so without a final attempt to retain for determinations of $CO_2$ a certain significance in the appraisement of the healthfulness of air. Their new interpretation was to the effect that the $CO_2$ percentage is proportional to the amount of deleterious organic matter, and for many years this view prevailed. It is still believed by some that an increase from the normal to 10 parts of $CO_2$ per 10,000 parts of air indicates a degree of organic pollution which is dangerous to health. More recent work definitely shows, however, that this view also must be abandoned, and there remains for $CO_2$ analysis only the secondary value that it indicates, in a readily measurable way, to what extent the inside air is being mixed by ventilation with pure air from the outside. However free this dilution may be, the atmosphere may still be deleterious to health and comfort unless certain other properties of it are incidentally altered.

This interpretation of the value of $CO_2$ analysis naturally leads to a consideration of the next possibility, namely, that the air in confined spaces is contaminated by the accumulation of organic poisons derived from the exhaled air of the persons living in it. It is many years ago now since experiments apparently proving this hypothesis were published. These consisted in placing small animals, such as mice, in a series of glass vessels connected together in series by tubing. Air was sucked through the series so that the animal in the second vessel received air that had been polluted by the animal in the first one, and the third by the first and second, and so on. It was found that the animals in the last one or two vessels of the series died after some time, whilst the others remained perfectly healthy. The original experiments were very improperly

performed, however, and their repetition with proper care to keep down the $CO_2$ below a concentration which *is* fatal, such as 10 or 15 per cent., has not afforded any evidence that organic poisons were contained in the air.

Some of those who sought for evidence in support of this hypothesis, did experiments that border on the ridiculous, but yet it is important that they be referred to here, since they are sometimes quoted as being trustworthy. These experiments consisted in collecting the condensed vapours of expired air and then inoculating small animals hypodermically with some of the condensed liquid. About 1 c.c. was found to kill a mouse, and, no wonder, since this would mean an injection of something like 5 kg. into a man of average weight. Everyone knows what the injection of so much water would do. Even distilled water is highly toxic in much less amounts when it is injected subcutaneously, and when we add the fact that this condensed vapour was contaminated not only with various salts, but also with bacteria, the result on the mice becomes utterly meaningless.

During more recent years the attempt has been made to resuscitate the old hypothesis by supposing that the toxic substance is of the nature of a volatile protein. When the proteins of one animal are introduced, even in very minute quantities, into another animal in any other way than through the alimentary tract—for example, by being absorbed through the lungs—they set up in the body a peculiar condition, in which the animal becomes so very sensitive to that particular protein that when another minute quantity of it enters the body a serious poisonous reaction which is often fatal results. The phenomenon is called anaphylaxis. It was supposed that in a milder form a reaction of this type was responsible for the toxic influence of vitiated air. As proof for this hypothesis experiments were performed in which a man breathed through a filter of glass wool (to catch any saliva) into a cooled vessel, and the condensed vapour was then inoculated in appropriate dosage into guinea pigs, so as to sensitize them, and a month or so later the animals were inoculated with a minute trace of human blood serum. The injected animal showed decided symptoms of anaphylactic shock, whereas other animals not previously sensitized were unaffected by the injection of the same amount of serum. Such results taken by themselves did seem to afford substantial support for the new hypothesis, but it is almost certain that they depended on contamination of the condensed vapour by traces of saliva which it is impossible to keep out by any kind of filter. This saliva con-

tains traces of soluble protein (mucin) which had been responsible for the anaphylactic reaction. The symptoms are, however, entirely dissimilar from those of a vitiated atmosphere. Hay fever and the reaction which some persons show when near to horses may be due to anaphylaxis, but the symptoms are not at all like those of persons breathing polluted air.

Once and for all, the toxic theory, as we may call it, both in its new and its old form, is disproven by a very simple series of experiments performed a few years ago by Leonard Hill, Flack and others. These observers kept rats and guinea pigs in deep boxes so that they were huddled together in a very poorly ventilated place, the atmosphere of which indeed often contained 1 per cent. of $CO_2$ —ten times more than the legal limit. The animals lived and thrived for months, although they must have been breathing air which was highly contaminated by the supposed volatile proteins. Not only did the animals show no symptoms while in the box, but they failed to exhibit any anaphylactic reaction when, after some time, they were inoculated subcutaneously with the serum of animals of the other species with whom they had been in cohabitation. This was really a most excellent test of the anaphylactic theory because there are probably no two animals in which anaphylaxis is more pronounced than in the rat and guinea pig. The only things that were found to be of importance in maintaining the animals in a thriving condition were cleanliness and plenty of food.

By an eliminative process we are gradually approaching the correct solution of our problem, but before we proceed to consider this, it may be well to remark that the odour of polluted air has nothing whatever to do with its unhealthy influence, except in so far as it excites disgust and puts one off his appetite. Indeed, one very soon becomes so accustomed to these odours that they fail entirely to be sensed after a short period in contact with them. Their influence is entirely psychological. In many trades and occupations people are constantly exposed to odours that are almost unbearable to one who is unused to them, and these people are perfectly healthy, and, indeed, do not complain at all of the smells.

We have so far considered in what is approximately their chronological order the various hypotheses that have been brought forward to account for the harmful influence of vitiated atmospheres. We have done this mainly in order to correct any false conclusions that may still exist in connection with the subject.

And if further evidence be demanded to justify this position there is one crucial experiment which once and for all shows that

changes in the chemical composition of the atmosphere has no relationship whatsoever to the unhealthful influence of vitiated air. This experiment is all the more convincing because it was performed on healthy young men. In its simplest form it consists in crowding as many persons as possible into an air tight cabinet, provided with an electric fan, and with the necessary apparatus for measurements of the physical and chemical condition of the air. In describing the results of this experiment, I cannot do better than quote from Leonard Hill, who, though not the first to perform the experiment, has so greatly extended our knowledge of the science of ventilation during recent years.

"After 44 minutes the dry-bulb thermometer stood at 87°F., the wet-bulb at 83°F. The carbon dioxide had risen to 5.26 per cent. The oxygen had fallen to 15.1 per cent. The discomfort felt was great; all were wet with sweat and the skin of all was flushed. The talking and laughing of the occupants had gradually become less and then ceased. On putting on the electric fans and whirling the air in the chamber the relief was immediate and very great, and this in spite of the temperature of the chamber continuing to rise. On putting off the fans the discomfort returned. The occupants cried out for the fans. No headache or after effects have followed this type of experiment which has been repeated five times." Long before the discomfort had become extreme the oxygen percentage became so low that matches would not light. The disinclination to smoke cigarettes was not noticed until some time after it was impossible to light them.

In other experiments of similar type the person in the cabinet was allowed to breathe outside air through a tube, but with no amelioration of the uncomfortable feeling, or a person outside the chamber breathed for hours the air inside it through a tube without suffering and discomfort. Clearly, therefore, neither the chemical nature of the air, nor the presence of toxic substances in it, has any relationship to its evil influence. But the experiment is not merely destructive of previously held hypotheses; it also points the way to the true solution of the problem, for it indicates that stagnation of air loaded with moisture has some very close relationship to the discomfort. It shows that a change in the physical, rather than the chemical properties of the air is the real cause of its deleterious action.

These changes can affect but one function of the body, namely, that of heat dissipation, and by so doing cause disturbances in the mechanism of heat control. This does not necessarily imply that

this disturbance is so great as actually to cause an increase in the body temperature, although this is very commonly observed in persons who have been for some time in crowded places. It indicates interference with a mechanism which is responsible not alone for proper heat regulation, but also for the maintenance of a proper relationship of blood supply to different parts of the body, and for toxic stimulation of the nervous system.

At this stage it may be well to digress for a moment to explain how the body temperature is maintained. To a certain extent the mechanism is exactly that of a radiator, the temperature of which depends, first on the rate at which the furnace is burning and second, on the cooling influence of the air in contact with the radiator. The physical properties of the air upon which the cooling depends are those which influence radiation, conduction and convection. Now, turning to the body, these processes come into play mainly at the surface of the skin, where, however, excessive loss is guarded against partly by the low conductivity for heat of the skin and of the subcutaneous tissue (fat), and partly by the fact that the blood supply to the skin is scanty compared with that of the deeper tissues. This causes the blood flowing in the skin to have a decidedly lower temperature than that in the tissues a few millimeters deeper. This relationship of superficial and deep temperatures is maintained by the action of vasomotor nerves to the blood vessels, and whenever the body is exposed to warmer air, the vessels of the skin become dilated so as to draft more blood from the deeper to the superficial vessels causing flushing of the skin. Flushing of the skin is therefore a normal reaction, but at the same time it is a warning that the heat-regulating mechanism is being put on a strain. But it is inadequate to account for all the heat loss, for man can withstand temperatures that are not greatly below those of his body, indeed, he can tolerate for some minutes temperatures that are higher. It is recorded, for example, that two observers exposed themselves for a short time in an oven in which a steak was cooking, and it is well known that certain miners work for considerable periods at very high temperatures.

Evidently some other mechanism independent of the cooling effect of air itself, and not acting in the case of a radiator, comes into play. This is evaporation, and it occurs at two places in the body; at the surface of the skin, where sweat is evaporated, and in the lungs where the expired air is saturated with water vapour. The physical factors which control the degree of heat loss by evaporation at these two places are not precisely the same. In the

case of the lungs the inspired air becomes saturated with water at body temperature, and the amount of evaporation necessary to do so depends upon the amount of water already contained in the inspired air, that is, on the absolute humidity; the lower this is, the more water will it require to effect saturation.

In the case of the evaporation of sweat, the amount of moisture vapourized from the body depends on the relative temperature and the humidity of the atmosphere.

It may be well to digress for a moment to explain what is meant by these terms relating to humidity. By absolute humidity is meant the weight of water contained in a unit volume of air. This increases greatly with the temperature of the air; thus at 70°F. one cubic foot of air contains 7.91 grs. of water. Relative humidity, on the other hand, means the degree to which the air is saturated with moisture at each temperature; thus, a relative humidity of 75 at a temperature of 70°F. means that it contains 75 per cent. of the total of 7.91 grs., which, it would contain if saturated at this temperature.

Now, inasmuch as the air after expiration is at about the same temperature as the body, and is practically saturated with moisture, it follows that the main factor influencing loss of heat by this means will be the amount of moisture actually present in the inspired air. If this be nearly at body temperature, 97°F., and dry, each 100 c.c. will take up .00413 gm. water in the lungs; if it be at average room temperature (say 68°F.) and dry, it will take up just exactly the same amount to become saturated with vapour. Some heat, it is true, will also be required in this latter case to raise the temperature of the air itself, but this is small when compared with that required to hold the water as vapour, since air warms up easily, or, to use the scientific term, has a low specific heat.

The amount of heat dissipated from the body of man by this means in an ordinary living room is about 10 per cent. of the total loss, but it becomes relatively much greater when the air is dry, and especially when the breathing is increased. On the other hand, when the humidity of the outside air is great and the temperature high, little heat loss occurs through this pathway.

Under ordinary conditions of living somewhat less heat is lost by evaporation of the sweat, and the factors which mainly determine it are the temperature and the relative humidity of the atmosphere, provided the temperature be above a certain level.

It is in connection with this phase of the subject, more than any other, that many people find it difficult to understand the true sig-

nificance of relative humidity to the well-being of the body. The difficulty depends on the fact that the relative humidity has an opposite influence at low and high temperatures. In the former case it increases the conductivity of the atmosphere for heat and has a cooling influence, and in the latter it interferes with the evaporation of sweat, and has a heating influence. Below about 65°F. the cooling effect of moist air is prominent because there is little sweating, therefore a cold, wet atmosphere is chilling—it conducts heat away. At about 70°F., the cooling effect of air disappears and sweat occurs. The evaporation of the sweat now causes cooling, the degree of which varies inversely with the relative humidity. Between these two temperatures, *i.e.*, 65 and 70, there is a range in which humidity has little influence—a neutral region. The influence of high relative humidity on bodily comfort at temperatures above the neutral temperature becomes very marked indeed at 85°F., and a relative humidity of 90%, for example, very serious symptoms appear in a few minutes, when there is no movement of the air.

Relative humidity and temperature alone are not, however, the only physical conditions to be considered. Another is the movement of the air, for even under the unfavourable conditions just cited immediate relief is afforded if an electric fan be started, as it will be recalled was the result in Hill's experiment. The temperature in the cabinet was 87°F. dry bulb, and the relative humidity very high indeed when the symptoms became serious; by turning on the fan these conditions of the atmosphere were not altered, but the students immediately felt comfortable. The movement of the air enables it, though nearly loaded to its full capacity with moisture, to carry away considerable quantities in small loads.

The wearing of clothes greatly affects the rate with which these changes occur. The clothes act as barriers preventing the movement and exchange of air around the body. The garment next the skin entraps a layer of air which is more or less at the same temperature as the skin, and which soon becomes saturated with moisture at that temperature. Between the inner garments and those over them other layers of air are entrapped, each one being at a somewhat lower temperature and containing less moisture than the one inside. These layers of air, therefore, form stepping stones as it were between the extreme conditions at the surface of the skin, and the environment of the clothed body. Obviously if the layers of air next the skin are to be renewed at such a rate that they remain cooler than the skin and unsaturated with moisture the clothing must be adjusted to suit the outside conditions.

There is every reason for believing that it is because of interference with these processes that improperly ventilated and overcrowded places are uncomfortable. The moisture exhaled and evaporated from the bodies soon raises the relative humidity so that heat loss is retarded from the skin, and the heat that is actually given off raises the temperature so that loss from the body by radiation and convection becomes suppressed. As the temperature steadily rises, the air takes up more and more moisture, with the result that less and less heat comes to be lost from the lungs in saturating the expired air with vapour. The physical conditions of the environment become unsuitable for the physiological mechanism of heat loss, although meanwhile heat production goes steadily on. The body furnaces are not damped down in proportion as the loss of heat diminishes, and the consequence is a rise in the temperature of the blood—a mild fever. Now it is well know that the cellular activities which, taken together, make up the life process of the body are extraordinarily sensitive to change of temperature; their chemical processes become changed, they demand more oxygen, they fail to get rid of effete products properly, substances which have no action on them under the ordinary conditions of temperature become toxic, and so forth. A highly abnormal internal environment therefore becomes created around the living tissues of the body.

But short of a measurable rise in the temperature, improperly ventilated places cause reactions in the human body that are responsible not only for the discomfort which is experienced, but also for a lowering of resistance to infections. These reactions are due in the first instance to alteration in the temperature differences between the skin and the underlying tissues. Normally, as has been remarked before, this difference maintains at the skin a constant stimulation of the thermic nerves, and this stimulation is important in maintaining the tone of the nerve centres. The nerve cells that control the functions of the body do not originate impulses; they only act when other afferent impulses arrive at them. There are many varieties of stimuli which may excite these afferent impulses, but none more important than those which excite the heat nerves of the skin. This stimulation depends on changes in the rate at which heat is passing through the sense organs in which these nerves terminate. It is necessary to emphasize that it is the rate of change that acts as the stimulus and this depends on changes set up between the deep and superficial temperatures. When the skin vessels become dilated so large a volume of blood reaches

the surface that this difference becomes slight and the thermic receptors are not stimulated. There are many practical applications of these principles, thus it is because of stimulation of the thermic skin nerves that cold baths have a bracing effect, that the open air treatment, as in tuberculosis, tones up the body and enables it the better to hold its own against the tubercle bacillus and that sleeping out of doors is the best tonic for maintaining good health. In open air treatment it is true that the body is closely wrapped up—that is essential—but this does not eliminate the cooling influence, for not only does the cool air play on the exposed face and hands, in the skin of both of which the thermic nerves are very sensitive, but it acts also on these nerves in the skin, under the clothes, for the clothes merely serve to regulate the rate of cooling. This still goes on very much more than it would with much less clothing in an atmosphere that is stagnant, hot and humid. Open windows in bedrooms are never so healthy as open air porches, because there is no draft. It is the draft that is important. Naturally it must be regulated so that it is not restricted to one part of the body only—that obviously would introduce conditions to which the body is unaccustomed—it must blow equally all over. There is probably no greater fallacy in popular hygiene than that drafts are dangerous. Like all good and desirable things they become so only when they are improperly used. When a person, overheated by being in a hot atmosphere, is suddenly subjected to a restricted draft of course there is danger that the sudden change of conditions, affecting one part of the body only, will cause vascular disturbances that may be undesirable, but if the conditions be properly controlled, drafts are the healthiest things and the best tonics.

This brings us to a problem in ventilation that is attracting very considerable attention at the present time, namely, the relationship between ventilation and infections. It is a common experience not only that ordinary colds, but more serious infections as well, can be directly traced to some unsuitable condition of ventilation; such as sudden exposure to a draft while overheated, or going out into a cold, damp atmosphere from an overheated room. What is the reason for the infection under these conditions? At the outset we must recognize that all these conditions, colds, catarrhs, bronchitis, just like the more acute infectious diseases like diphtheria, pneumonia, cerebro-spinal fever, etc., are due to micro organisms, and the question therefore is why should unfavourable ventilating conditions so frequently be the immediate cause of the attack.

There are two methods by which the infection might occur. First, by a great increase in the number of organisms in the air, and, secondly, by a lowering of the resistance of the body towards the organisms, which would not then require to become increased in numbers. The former method is usually known as mass infection, and there can be no doubt that it is very common, perhaps, indeed, it is the commonest cause for infection. The organisms, of course, come from infected individuals, who add them to the atmosphere in the exhaled air, particularly when this is forcibly discharged as in coughing, or sneezing, or even in speaking.

I need recite to you only a few observations to convince you of the importance of this factor. If the mouth be rinsed with a culture of some readily recognizable organism not commonly present in detectable amounts in the atmosphere, and the person, standing in front of a row of plates each containing some culture medium upon which the organism will grow, then speaks at ordinary pitch, the plates after proper incubation develop colonies of the organism, those nearest the speaker having most, but even those at a distance of several feet also showing them.

A serious problem in zoological gardens has been to keep animals that are highly susceptible to tuberculosis free from this disease. The higher apes, for example, inevitably succumb to this disease, being infected by the bacilli exhaled by persons standing in front of their cages, many of whom harbour the tubercle bacilli, though they may not show any of the symptoms of tuberculosis. Now it has been found that if glass screens are erected in front of the cages the animals remain almost free from the disease. The lesson which these and many other similar observations teach is that we should avoid as much as possible getting in the direct path of the exhaled air even of apparently normal individuals, and when an infectious disease is prevalent, it should not be considered rude in conversation to stand aside a little and even to hold the hand, or better still, a newspaper, before the mouth. This may seem impracticable advice. But why so? In the light of such convincing experiments as those which have been cited above, and they are only two in a multitude—why should people not be more careful about being infected, especially during dangerous epidemics such as that of influenza. The thing to bear in mind is that a person may be harbouring the deadly bacteria and yet be in perfect health. The bacteria that are inocuous to him may find in another person more favourable conditions for growth, and so produce the disease.

But mass infection does not suffice to explain the cause for the onset of attacks of many conditions that are nevertheless fundamentally due to bacteria, such as ordinary colds. These can frequently be traced to some chill, or wet feet, or exposure to sudden change in temperature. In such cases it is believed that the bacteria are present on the mucous membranes of the upper respiratory passages, but that they remain inactive because of the normal protective influences which exist on these surfaces. So long as the blood supply is normal, these protective influences are adequate to protect the body from invasion, but if this should become curtailed, then the bacteria become active and set up pathological processes. Evidence favouring this view has been obtained by several recent investigators by finding that the blood supply of the upper respiratory passages becomes decidedly curtailed when the surface of the body is cooled. For example, Leonard Hill and Muecke some years ago examined with a speculum the mucous membranes of the nose under various conditions, particularly out of doors, and in rooms which were ventilated and heated to an average degree. Out of doors the mucosa was pale and taut, and when touched by a probe did not show any pitting. This is the normal condition. Indoors it was common to find the membrane decidedly swollen, flushed with blood and covered with thick secretion, and when a probe was pressed on it a depression resulted lasting for some time. In one case that was frequently examined during these observations there was a deflected septum which only partly blocked the nasal passage on one side when the person was outside, but which did so completely under unfavourable conditions of ventilation. It is this swelling of the nasal mucosa and probably of that of the cavities which extend upward from it on to the forehead that causes the sense of stuffiness and probably also the headaches which are common in crowded, over-heated places.

The conditions found to bring about these changes with greatest certainty were when the feet were cold and the air round the head was warm, conditions which are just exactly the opposite of those obtaining out of doors. Here the head is usually more quickly cooled than the feet, because convection currents of cool air play around it freely, whereas next the ground the air is more stagnant. Besides, if the sun is shining, the earth becomes heated by absorbing the heat. The temperature as registered by a thermometer, either wet or dry bulb, may be the same at the feet as at the head. It is not this that counts, however; it is the rate of cooling which is dependent, mainly, on the movement of the air. Now, in a poorly

ventilated room, such, for example, as one heated by a stove, or even by radiators, and in which there is no movement of air, the feet become colder than the head, and it is under these conditions that the nasal membranes become swollen. Leonard Hill, to illustrate the importance of these principles, cites an interesting observation which he made in the House of Commons. In the main chamber "the ventilating current is driven up through the floor in such a way as to cool the members' feet, while their heads are exposed to more stagnant air. Cold feet and stuffy heads result—just the wrong conditions for legislators." The thermometer shows a uniform temperature, but the Kata thermometer, which we will describe shortly, shows the cooling rate to be 40% greater at the feet than at the head level. Hill states that he always experienced obstruction of the nose, because of his deflected septum, when he sat on the benches, and that this disappeared when the air coming through the floor was cut off and the air was introduced at the head level. The cause for these changes is not cold feet alone. It is the combination of cold feet and hot head. Out of doors, it is well known, that any one may stand with cold feet for hours without any risk of catching cold, but then the head is really cooling as fast as the feet, because of convection currents.

The ideal system of warming a room is to supply radiant heat near the floor level; open fires, properly flued modern gas fires, and electric heaters at floor level are the best methods to attain this. Steam heated radiators, especially if they are provided with vapourizers, are not desirable methods for heating unless the air of the room is frequently changed at high levels.

Suppose now the person subjected to conditions which cause the mucous membrane to become swollen and congested should go outside, then the membrane at once becomes pale because the blood vessels constrict, but for some time it remains swollen and boggy and continues to show pitting with a probe. It is while in this state that it offers favourable conditions for the growth of bacteria. The membrane is swollen and covered with secretion, and the blood flow is cut down. The natural defensive agencies that are normally carried by the blood do not succeed in combating the multiplication of the bacteria in the swollen membrane. After some time out of doors the blood supply returns because it is required to warm up the cool air, but this reaction does not occur before the mucosa has regained its normal condition.*

*The congestion of the mucous membrane brought about by warm, moist air, does not probably depend on dilatation of the small arteries—entailing increased flow of blood, but rather on dilatation of the capillaries, and therefore a stagnation of blood.

The protective influence of a rapid blood flow through the nasal membrane is possibly the explanation of the relative immunity from infectious colds of those who work in air containing irritating gases, such as workers in various kinds of chemical factories. Even the irritation set up by coal dust may, by similar methods, afford some protection against infection by the tubercle bacillus—for phthisis is relatively infrequent amongst coal miners. The supposedly antiseptic action of ozone is probably due to a similar irritating effect. Any benefit that may be derived from its presence in the atmosphere cannot otherwise be explained. It is possible that a useful prophylactic practice to avoid infection, such as that of influenza, would be to stimulate the nasal mucosa at intervals by snuff, but this may be an unwise suggestion.

After becoming acclimatized to outdoor conditions the nasal mucous membrane is in a much more favourable condition to withstand infection than indoors, because of the very rapid blood flow that is necessary in order to supply heat with which to warm up the inspired air. This more rapid blood flow, and the freer flow of lymph which accompanies it, is reinforced by increased secretion, which assists to wash away invading bacteria. Mass infection being equal inside and outside, the animal body can withstand it much less satisfactorily in the former case.

These observations on the reactions of the respiratory membranes to atmospheric conditions have been confirmed by other investigators. Thus Cock (H. Girard Cock, Tr. Am. Laryngol. Rhinol. & Otol. Soc. June, 1915) caused persons to breathe forcibly through the nostrils on to a mirror surface, and then marked on it with a wax pencil the outlines of the moisture deposited. Although the extent of the outlines varied somewhat with the depth of breathing, they afford a general estimate of the width of the air passage. It was found that there is marked reduction in the nasal air passage in a warm room. Winslow (Winslow, C.E.A., New York Commission on Ventilation) also sums up the observations (150 in number) made on this aspect of the problem, in the following words: "Ordinarily it was found that heat causes a swelling of the inferior turbinate of the nose, tending to diminish the size of the breathing space, increased secretion and reddening of the membranes. The action of cold is, as a rule, just the opposite."

Many other observations bearing on the relationship between chilling and immunity to infection have been recorded, but it would take us beyond our subject to discuss them here. Because of their accuracy and the excellent control of possible fallacies it is import-

ant, however, to say something about the recent investigations of Mudd and Grant (Mudd, S. and Grant, S. B. The Journ. of Medical Research, XL., p. 53, 1919). These observers measured the temperature of the mucous membranes of the palate, tonsils and pharynx by means of thermo-couples before and during application to the skin of cold towels, or while cold air from a fan was allowed to play on it. A rise in temperature would indicate that the part had become more vascular, and a fall, the contrary. That this interpretation was the correct one was confirmed by direct inspection of the degree of flushing (redness). It was found that chilling the body surface immediately caused a fall in the temperature of the mucous membranes which could not be accounted for by any accompanying change in blood pressure, or, entirely at least, by changes in respiration or by lowering of the temperature of the blood. The conclusions are "that chilling of the body surface causes reflex vaso-constriction and ischaemia in the mucous membranes of the palate, faucial tonsils, oropharynx and nasopharynx."

And now the final question presents itself, what are the ideal conditions of ventilation? It is a most difficult question to answer, and one over which at present several large commissions are at work. Indeed, most elaborate experiments have been planned and undertaken to throw light on the question. The observations of the New York Commission on Ventilation, by Mr. Watt, in the Graham School in Chicago, are among the most important in this country, and, of course, they interest us much more directly than those conducted on the other side, where the climatic conditions are fundamentally different.

The observations have been made very largely on properly selected groups of school children, taught in class-rooms with different ventilating conditions. Attention is directed to the general efficiency of the pupils and the condition of their health. The temperature, and the humidity of the air are the physical conditions of the atmosphere which have been more particularly studied, but a great deal more work must be done before any definite conclusions can be offered. It appears, however, that for schoolroom air a temperature of 65-68°F., with a relative humidity of 45-60% is the optimum. To maintain these conditions throughout the period a class occupies the room, usually requires, in this country at least, the addition of a considerable quantity of moisture to the ventilating air. The air of most of our school rooms in winter errs on the side of being too dry, for under these conditions the mucous mem-

branes suffer injuriously. An excellent summary of the various authoritative conclusions with regard to the optimum conditions of ventilation for class-rooms is given by Burnham in the *Pedagogical Seminary* (Burnham, W. H., *The Pedagogical Seminary*, 1919, XXVI., p. 311).

Although the present review does not venture to discuss the methods that are employed for the measurement of the various physical properties which have to be considered in gauging its influence on health, nor the engineering problem of how ideal conditions may be maintained, it may not be out of place to mention, in connection with the former of these, that the physical property to which most attention should be devoted is the cooling power. This cannot be done by reading an ordinary thermometer, for this instrument only registers the temperature of the piece of wood and of the wall against which it is hung. It registers the same whether the air is dry or moist, or whether it is stagnant or moving. Somewhat more information regarding cooling power is afforded by readings of a wet-bulb thermometer, an instrument in which the bulb is kept constantly moist, so that evaporation occurs from it. This evaporation tends to cool the thermometer, in proportion to its rate, and since this is dependent mainly on the degree to which the air can take up more moisture, we can tell by the use of a formula or tables the relative degree of humidity of the air. Still this does not tell us the real degree of cooling which the atmosphere can bring about. It does not adequately register the cooling which is dependent upon movement in the air, the so-called convection currents. To afford this information Leonard Hill has invented what he calls the Kata thermometer, by which the rate of cooling is directly measured. The instrument consists of an alcohol thermometer with a relatively large bulb, and with the scale registering between 105°F. and 90°F. It is placed in warm water at about the former temperature, and is then removed, and the time required for the temperature to fall from 100°F. to 95°F. is measured by means of a stop watch. This time divided by a factor determined for each instrument, and written on the stem, gives the actual amount of heat in millicalories per square centimetre per second which would be given off from, say, the surface of the human body, under similar environmental conditions. Hill and his associates have shown that much important information concerning the cooling power of the atmosphere can be gained in this way, which cannot be gained by any other.

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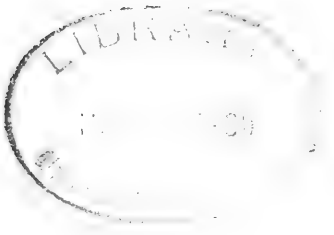

No. 33: OBSERVATIONS ON THE GLYCOGEN CONTENT OF CERTAIN INVERTEBRATES AND FISHES, BY L. G. KILBORN AND J. J. R. MACLEOD

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# OBSERVATIONS ON THE GLYCOGEN CONTENT OF CERTAIN INVERTEBRATES AND FISHES.

By L. G. Kilborn and J. J. R. Macleod. (From the Marine Biological Station, Nanaimo, B.C., and the Department of Physiology, The University of Toronto.)

(*Received for publication* 27*th December* 1919.)

Throughout the animal kingdom, from the Amœba to Man, carbohydrate is known to be an important constituent of the organism. It is most characteristically represented within the cells by the polysaccharide glycogen, and in the circulating fluids by glucose. In the higher vertebrates the percentage amount of glycogen in the different organs and tissues varies considerably, being usually greatest in the liver. The amount is, however, closely related to the time of feeding and the nature of the food. In the circulating fluids of the animal, on the other hand, there is apparently a tolerably constant percentage of glucose, if the temporary increase which follows immediately upon the ingestion of food (the postprandial rise) be discounted.

Here and there in the animal kingdom, however, it has been asserted that very little, if any, glycogen (certain molluscs) or glucose (Selachians) is present. If this should prove to be the case, it would mean either that some other carbohydrate is substituted for glucose and its polysaccharide—such as a pentose,—or that metabolism proceeds in these animals in the absence of any of the higher carbohydrates. Scrutiny of the published researches upon which these generalisations depend shows that the methods employed have been very unequal in value both qualitatively and quantitatively. For the detection of glycogen the microchemical reaction with iodine has been extensively used, and for the quantitative determination of this substance usually the somewhat uncertain method of Brücke-Külz; it is only here and there that the more certain method of Pflüger has been employed. For the detection and measurement of the glucose it has been usual to utilise the reducing power, after precipitation of the proteins by various methods.

In consideration of these facts, it was thought advisable to seize the opportunity afforded by several weeks' residence at the Marine Biological Station (situated on the east coast of Vancouver Island, at Nanaimo, B.C.) to determine by standard methods the relative amounts of the above-mentioned carbohydrates in selected varieties of marine animals. In planning such an investigation it was recognised that it is decidedly risky

to assume that methods for the isolation and determination of glycogen and soluble reducing carbohydrate, which have been shown to be reliable for higher land mammals, must also necessarily be so in the varied conditions met with in the lower marine animals. The chemical structure of the circulating fluids and of the tissues might, for example, be so far different as to interfere with the proper removal of the proteins prior to estimation of the glycogen or glucose. Notwithstanding this possible source of fallacy, it was thought that the first step in the investigation should consist of determination of the above substances by the accepted standard methods, reserving for future research a more detailed investigation of the causes for deviation in the results.

A general review of the work bearing on the distribution of glycogen in the animal kingdom is given by Pflüger (1) (up to 1903) and by Biedermann (2) (up to 1911).

Much of the work referred to in these reviews is microchemical in nature, and may for the present be disregarded. Of the work in which glycogen was isolated by chemical methods, the following references have more or less bearing.

Amongst the Protozoa, glycogen has been isolated by the Brücke-Külz method in Infusoria (Barfurth (3)), particularly in a culture of Glaucoma scintillans. The isolated glycogen gave the characteristic reaction with iodine, and yielded a reducing substance after hydrolysis with mineral acid. The starch-like granules, called paraglycogen, which have been described in the protoplasm of certain of the Infusoria (Gregarina), are apparently not ordinary glycogen, for although they give a brown reaction with iodine, they are insoluble in cold water, and are not hydrolysed to reducing sugar by saliva. By prolonged hydrolysis with weak acid, however, reducing substance is produced (Bütschli (4); see also Maupas (5)).

The so-called "Glanzkörper," found particularly in Pelomyxa, are also often considered as being closely related to glycogen. They are readily formed in the cell when the animal is fed with polysaccharides (starch, cellulose), they give a reaction with iodine like that given by glycogen, and reduction occurs after hydrolysis of the bodies of animals containing them. Apart from these observations, there appears to be no chemical evidence that the bodies are really glycogen (Greeff (6)).

Biedermann, in summing up the work on the Protozoa, states that the digestion of starch proceeds in these animals much in the same way as in the Metozoa, and that storage of the digested starch in the form of glycogen certainly occurs in Pelomyxa and often in the Ciliata and Gregarina (loc. cit., p. 386).

In Euglena, which represents the highest of the Flagellata, a substance called paramylon has been extensively studied by microchemical methods, and has been found to vary greatly in amount according to the pabulum upon which the Euglena is grown, and whether or not it is associated with

chlorophyll. When chlorophyll is present in the cell paramylon is abundantly formed and can be dissolved out by weak alkali and precipitated by acid alcohol. It gives no colour with iodine, is insoluble in water, and when hydrolysed by acid yields a reducing substance which ferments with yeast. Diastase is said not to digest this material, however (Bütschli (7)).

It is stated by Pflüger (op. cit.) that glycogen has been described as present in certain of the Echinodermata (asteroids, holothurians) and in sponges. It has been detected by microchemical methods in the eggs of insects and molluscs. As much as 2 per cent. of glycogen, apparently identical with that prepared from rabbit liver and from oysters, was isolated by Harden and Young (8) from washed pressed yeast.

Taking the observations as a whole, it is certain that polysaccharide material, which is more or less closely related to glycogen, is a common constituent of the cells of the lowest animals as well as of such plants as yeast, which do not synthetise sugars through the action of chlorophyll.

From certain Ascaridæ and also from Tænia, Kobert (9) was able to prepare a strongly opalescent watery solution reacting with iodine. On standing the glycogen became digested, although the extract did not cause digestion of added starch. This would seem to point to the existence of a specific glycogenase. Weinland (10) found that about one-third of the weight of dried Ascaris consisted of glycogen; in Tænia even a higher amount, namely, one-half of the dry weight, consisted of this material. In Distoma hepaticum also a large amount of glycogen was present. It is interesting to note in passing that this glycogen was found quickly to disappear when the worms were kept under anaerobic conditions. Glycogen is also present in considerable amount in the earthworm (Lesser (11)). In the so-called chlorogogen cells which surround the alimentary canal very large amounts of glycogen have been described: the leucocytes in the body fluids also contain it, but it is said to be absent from the muscles (Cuenot (12)).

A considerable amount of attention has been paid to the carbohydrate present in the digestive gland (liver) of various Mollusca. Biedermann gives an adequate review of the work. Of particular importance in the present connexion is the work of Barfurth (cf. p. 342, op. cit.), who found in air-breathing gastropods after feeding for one to three days with bread from 3·4 to 6·4 per cent. in the liver and 3·3 per cent. in the foot. He concludes that the liver of gastropods plays almost as important a part in storing glycogen as does that of mammals.

In those snails (Helix) in which there is relatively a large amount of connective tissue, the glycogen was found to be stored mainly in the plasma cells that are present in this tissue, whereas in snails (Limax) in which there is little connective tissue the plasma cells soon become filled and the glycogen is present mainly in the columnar epithelial cells both of the intestine and of the biliary passages (Barfurth, op. cit., p. 328).

These observations are of interest in connexion with the observation first made by Claude Bernard, and subsequently confirmed by Barfurth and Biedermann, to the effect that the contents of the stomach and upper portion of the intestine, after they have been partially digested, move in and out of the liver passages. Absorption occurs through the cells both of the intestine and biliary passages, and when it is completed the fluid left in the stomach is colourless, though at first it was coloured by the secretion of the liver. It is important to note these facts in connexion with the present investigation, because they indicate that we must be careful not to conclude that any glycogen found in the liver of molluscs is necessarily present in this organ as such. As a matter of fact, several investigators (Frentzel, Röhmann (13), Bottazzi (14)) have been unable to detect any glycogen either by microchemical or biochemical methods in the livers of Aplysia and Arion. The two observers last mentioned have, independently, found in Aplysia (the sea-hare), in place of glycogen, a pentose-yielding substance (pentosan), the precise identity of which is uncertain. Röhmann thought this pentosan to be derived from undigested residue of the food of the animal, which consists of an Alga (Ulva lactuca) and which contains pentosan as well as starch. The residue finds its way into the biliary passages as described above. Bottazzi agrees that it is derived from the pentosan of Ulva, but believes that it has become partly broken up so as to form an acid substance (acide pentosique) which takes the place of mineral acid, which is absent in these molluscs.

M. Henze (15) could not find a trace of glycogen or of water-soluble carbohydrate in the muscles or liver of Octopods. Some evidence was obtained, however, that a glucoprotein may represent the carbohydrate storage material in these animals.

Further attention has been paid by Henze and Starkenstein (16) to the supposed absence of glycogen in the liver of molluscs. They point out that if this conclusion is confirmed it would indicate that, in so far as their carbohydrate metabolism is concerned, certain marine molluscs occupy a peculiar position in the animal scale, glycogen being apparently present in all others. They show that a certain error may be incurred in estimating glycogen in the organs of sea animals on account of the presence of the salts of sea water, particularly Mg. The $Mg(OH)_2$ which is formed with KOH adsorbs some of the glycogen, and when alcohol is subsequently added a precipitate is formed from which boiling water does not dissolve out all the glycogen. These authors also point out that an error of another nature may be incurred because of the presence of glucosamin derivatives and of pentosans, unless the tissue be treated with the strong alkali for a sufficient length of time to ensure the entire destruction of these substances. This matter will be referred to again later. It is recommended by Starkenstein and Henze that the above-mentioned sources of inaccuracy be circumvented by prolonged heating with KOH,

then adding only one volume instead of two of alcohol to precipitate the glycogen, and finally hydrolysing the alcohol precipitate directly without first of all dissolving it in water. They offer no experimental proof, however, that their method is any more accurate than the original method of Pflüger. They found as much as 1·25 grm. glycogen in the liver of Aplysia lenescona.

Among the Crustacea it was found by Hoppe-Seyler (17) that there was still some glycogen in the liver of 5–6-year-old crayfish (Flusskrebsen) after starvation. Claude Bernard had previously found the amount to vary more or less in relationship to the time of moulting, being greatest just prior to this period. At the time of moulting Kirsch (18) found the entire body (of the fresh-water crayfish) to contain 0·82 per cent., whereas four months before this period it contained only 0·08 per cent.

Until recently very little information existed concerning the presence of glycogen in the fishes. That some at least is present in the tissues of marine fish had been shown by Cl. Bernard, Pavy, Brücke, and others. It was stated by Bernard that this glycogen is unusually resistant to the influence of post-mortem changes, and that it does not readily disappear during hunger. During asphyxia, however, the glycogen rapidly disappears.

Schöndorff and Wachholder (19) adequately review all these older investigations, and contribute numerous observations of their own in which the amount of glycogen, determined by Pflüger's method, in a large variety of fishes caught in fresh water is given. The percentage amount varied between 2·5 and 12·94. Prolonged hunger was found to cause considerable reduction in the amount of glycogen in such fish as the pike (Esox lucius), which remain active, but to cause only slight reduction in the carp (Cyprinus carpis) and other fish, which hide themselves away in the mud during winter. Post-mortem glycogenolysis did not appear to proceed as rapidly as in Mammalia. The estimations were made by measuring both the reducing and the rotating power of the hydrolysed glycogen precipitates, the close correspondence of the results obtained by the two methods indicating that the material is chemically identical with that present in mammalian tissues.

## Methods.

### Glycogen.

The organ or tissue was cut into small pieces, pressed between filter paper, weighed, and dropped into 95 per cent. alcohol, in which it was shipped from the station to Toronto, the journey occupying about a week.

It is possible in the case of one or two specimens of muscle (e.g. siphon muscle of clam) that there was relatively too small a quantity of alcohol entirely to prevent a certain amount of glycogenolysis. The muscle in

these cases was decidedly compact, and may not have been sufficiently cut up to ensure immediate penetration by the alcohol. This possible source of error was, however, not incurred in the vast majority of cases. The alcohol-preserved material was pressed between filter paper and again weighed. It was then heated for three hours with 60 per cent. KOH and the glycogen determined by Pflüger's method.

As already mentioned, Starkenstein and Henze state that glycogen determination by the Pflüger method is inaccurate in the presence of sea water because of adsorption of the glycogen by the $Mg(OH)_2$ and $Fe(OH)_3$ that are formed when KOH is added. This adsorbed glycogen after precipitation with alcohol does not become completely dissolved in boiling water.

By dissolving equal amounts of pure glycogen in sea water and in distilled water and then carrying out the usual Pflüger process with both, we have confirmed the observations of the above authors. We do not believe, however, that the organs and tissues of sea animals contain a sufficiency of salts precipitable by KOH to make any significant error in the glycogen determinations. This conclusion is based on the following observations :—

The liver of a rabbit was cut in small pieces and quantities of 20 grm. each were added to equal volumes of (a) sea water, (b) 0·9 per cent. sodium chloride solution, in which they were thoroughly ground in a mortar. To each of the resulting suspensions equal volumes of 60 per cent. KOH solution were added and the glycogen content determined by the Pflüger process. The following results were obtained: in (a) 0·675, (b) 0·784 per cent. Even in the presence of a very large excess of sea water—very much more than could be present even in tissue which has not been pressed between filter paper—the error incurred is not excessive.

In order to see whether any large yield of glycogen would be obtained when the alcohol precipitate was directly hydrolysed, 28 grm. of oyster (from which the excess of sea water had been removed by pressing between filter papers) was treated with KOH and the glycogen precipitated with alcohol and divided into two portions, *a* and *b*. The precipitate in *a* was dissolved in boiling water in the usual manner (i.e. on the filter paper, and the filtrate hydrolysed), whereas that in *b* was removed from the filter to a flask by a fine stream of water and directly hydrolysed. The following results were obtained: in *a*, 0·569 grm. glucose, in *b*, ·694 grm. glucose. There is therefore evidence that it is necessary to modify the Pflüger process to the extent that the glycogen precipitates are washed from the filter paper into a flask and directly hydrolysed. This procedure has not been followed in the present investigation, partly because Starkenstein and Henze's work did not come to our notice until the work was nearly completed, and partly because the precipitates in many cases were of such a nature that their removal from the filter

paper would have been practically impossible. It is considered that the error incurred on this account is insignificant, especially since very little of the salts of sea water could have been present in the tissue used for the estimations.

## Results.

### Glycogen.

These are given in percentages both of the original and the alcohol-dried material. Although neither result in itself is more than approximate because of the impossibility of removing all the adherent moisture or alcohol by means of filter paper, they are sufficiently close to be used for the purpose in view, namely, to determine the relative amounts of glycogen in the tissues of different animals.

The observations were made on specimens from the following groups of animals:—

The Echinodermata (Asteroidea).
The Mollusca (Lamellibranchiata).
The Arthropoda (Crustacea).
The Fishes (Elasmobranchii and Teleostomi).

### I. THE ASTEROIDEA.

Table I.—Percentage of Glycogen (as Dextrose) in the Hepatic Cæca of Star-fishes.

| No. | Species. | Weight of material (grm.). | Glycogen percentages | | Remarks. |
|---|---|---|---|---|---|
| | | | Calculated for original material. | Calculated for alcohol-preserved material. | |
| M | Pisaster ochrasea . . | 41·9 | 1·23 | 1·52 | 1 large specimen. |
| T | „ „ . . | 14·2 | ... | 0·268 | 2 small specimens. |
| $E_1$ | „ „ . . | 16·6 | ... | 0·478 | 3 „ „ |
| $H_1$ | Pisaster brevispinus . | 6 | ... | 0·232 | 1 specimen; liver very small. |
| L | Pycnopodia heliantoides . | 34·3 | 0·62 | 0·93 | From 1 specimen. |
| $I_1$ | „ „ . | 24·6 | ... | 0·60 | |
| S | Evastarius Troschelli . | 11·95 | ... | 0·90 | |
| $F_1$ | Luidia foliata . . . | 22 | ... | 0·461 | Mud star-fish. |
| $G_1$ | „ „ . . . | 18 | ... | 0·403 | |

The variable amount of glycogen found in these animals is probably dependent upon whether or not they have recently been feeding, the percentage of glycogen being plainly proportional to the size of the cæca. The glycogen precipitate in M was further examined. After redissolving in boiling water it was found to give a characteristic reaction with iodine, and on hydrolysis to yield a sugar which fermented readily with yeast.

## II. THE MOLLUSCA.

Table II.—Percentage of Glycogen in Various Parts of the Body of the Horse Clam (Schizothoerus Nuttalli).

| No. | Organ or tissue. | Weight of material (grm.). | Glycogen percentages | |
|---|---|---|---|---|
| | | | Calculated for original material. | Calculated for alcohol-preserved material. |
| A | Digestive gland . | 31·2 | ... | (a) 0·31 |
| N | „ „ . | 22·5 | ... | (b) 1·56* |
| F | Muscle of siphon . | 56·1 | ... | (a) 0·077 |
| Q | „ „ . . | 30 | ... | (b) 0·952 |
| C | Muscle of foot . . | 54·25 | ... | (a) 0·46 |
| P | „ „ . . | 19·4 | ... | (b) 1·70 |
| B | Adductor muscles . | 63·5 | ... | (a) 0·40 |
| O | „ „ . . | 20·6 | ... | (b) 2·67? |
| R | Posterior adductor . | 17·1 | ... | (b) 2·75? |

* Hydrolysed glycogen fermented with yeast.

The estimations marked (a) were made on clams that had been kept for some time (1–2 weeks) in a sack immersed in the sea at the wharf. Those marked (b) were made on clams that had been kept only a day or so after digging them up.

These observations were carried out on two batches of clams. After being dug from a sandy beach at low tide the clams were placed in a sack which was then immersed in the sea at the end of the wharf, specimens being removed from time to time for analysis. The clams of the first batch *a*, although receiving no food, remained alive in the sack for over two weeks, but those of batch *b* did not survive in the sack for more than a few days, and they disintegrated very rapidly after death. The estimations, the results of which are recorded above, of group *a* were made after one week in the sack; those of group *b*, on the other hand, were made within a day or two after the clams were collected. It is possible that variations in the state of the material accounts for the persistently higher percentages of glycogen in group *b* than in group *a*. In order to make a comparison of the glycogen in the various organs and tissues, it is necessary, therefore, to take the results of each batch, *a* or *b*, separately.

In batch *a* the largest amount of glycogen was found in the muscle of the foot, with very nearly the same amount in the adductor muscles. Decidedly less was present in the liver, and only a trace in the muscle of the siphon. More interesting results are those of batch *b*. In this case the adductor muscles contained a higher percentage of glycogen than we can find recorded for muscle. (The highest given by Pflüger in "Das Glykogen" is 2·44 per cent. This was found by Aldehoff in the glutæus maximus of the horse.) We are, however, not certain that it is typical glycogen that is responsible for all the high percentage found in the case of the adductor muscles. There are several reasons for this doubt: first,

the precipitate produced by alcohol did not settle as ordinarily, but required the addition of a considerable amount of sodium chloride to cause it to do so; second, on neutralising the hydrolysed solution of the precipitate and fermenting with yeast only a small quantity of gas collected, whereas the precipitates from the liver, when similarly treated, give a much larger amount of gas. We had not sufficient material with which to investigate this question further. These muscles are entirely different in structure from the muscle of the foot or siphon, being composed of large bundles of very pale substance.

There is also a high percentage of glycogen in the muscle of the foot, although this muscle can be used but seldom after the clam has assumed its more or less permanent position in the sand. The glycogen in the siphon muscle is decidedly less (0·95 per cent.); this muscle is used to hold the tubes open as well as to retract the siphon on the approach of danger.

It will be observed that a considerable amount of glycogen was found in the liver. This is of interest because of the belief already referred to (p. 320), that there is no glycogen in the liver of certain molluscs (Aplysia and Arion). That the material found by us was glycogen, as ordinarily understood, was shown by its general behaviour towards strong alkali, iodine, etc., and by the fact that the hydrolysed glycogen readily fermented with yeast and gave typical glycosazone crystals.

It will be recalled that several observers (Frentzel, Röhmann, Bottazzi) have averred that there is no glycogen in the liver of certain other species of the Mollusca. These workers found evidence of the presence of pentoses and methylpentoses, and they have suggested that polymerised forms of these may replace glycogen. Since they did not consider the likelihood that the pentoses were derived from inosinic acid or guanylic acid, the conclusions cannot be given much weight. As a matter of fact, it has been shown by Mr C. E. Berkeley, working in the biological station, that the pentose present in the tissues of closely related species is derived from one or other of the above-mentioned nucleotids.

The percentage of glycogen is notably different in the small crab (Cancer productus) and in the lobster. The difference is probably dependent upon feeding conditions. The lobsters were shipped from St Andrews, on the New Brunswick coast, to Toronto in moist seaweed, the first batch in September, when the weather was warm, and the second batch in November, when it was very cold. The difference in temperature does not appear to influence the results. The larger crabs were caught at St Andrews in November and transported to Toronto. They were almost dead when received. Although they contain much less glycogen in proportion than the smaller crabs, it will be noted that there is decidedly more in the liver and in muscle than in those tissues in the lobster.

A comparison of the glycogen content of different muscles in the lobster is of interest. In the muscles of the back and tail the average for four

## III. THE ARTHROPODA.

Table III.—Percentage of Glycogen in Various Parts of the Body of Crustacea (Cancer productus and Homarus Americanus).

| No. | Species. | Organ or tissue. | Weight of material (grm.). | Glycogen percentages | |
|---|---|---|---|---|---|
| | | | | Calculated from original material. | Calculated from alcohol-preserved material. |
| D | Cancer productus | Liver | ... | ... | 1·39 |
| $D_1$ | | „ | 7·37 * | ... | 0·37 |
| E | | Muscles | 24·65 | ... | 0·87 |
| p. 34 | Homarus . . | Liver | 35·64 | 0·78 | |
| „ 36 | | I. Heart | 2·37 | 0·91 | |
| | | II. „ | 1·94 | 1·42 | |
| „ 40 | | I. Muscle (tail) | 55 | 0·36 | |
| „ 41 | | „ (claw) | 50 | 0·17 | |
| „ 44 | | II. Muscle (claw) | 45·7 | 0·10 | |
| „ 48 | | II. „ (tail) | 42·6 | 0·32 | |
| 1 (p. 3) | Homarus I. . | Heart (of six lobsters) † | 7·32 | 0·85 | |
| (p. 4) | | Liver | 30 | 0·05 | |
| | | Muscle (back) | 20 | 0·31 | |
| | | „ (claw) | 20 | Trace | |
| | Homarus II. . | Liver | 20 | 0·13 | |
| | | Muscle (red claw) | 20 | 0·17 | |
| | | „ (white claw) | 20 | 0·17 | |
| | | „ (back) | 20 | 0·30 | |
| | Homarus III. . | Muscle (back) | 20 | 0·30 | |
| | Cancer irrotatus | Heart ‡ | 2·77 | 0·51 | |
| | | Liver | 24·47 | 1·00 | |

* Weight after preservation in alcohol.

† The hearts of six lobsters were used for this determination. The weight of the animals and of the heart in each specimen was as follows: (1) 2000 grm. and 1·95 grm., (2) 1650 grm. and 1·35 grm., (3) 1150 grm. and 1·00 grm., (4) 1150 grm. and 1·02 grm., (5) 1100 grm. and 1·00 grm., (6) heart 1·00 grm.

‡ The hearts of five specimens were used.

lobsters was 0·32 per cent., being remarkably close to this figure for all of the animals. The pale muscle of the claw gave an average for three lobsters of about 0·15, only a trace being found in that of a fourth animal. There is therefore decidedly more glycogen in the tail muscle. Of greater interest are the results obtained for the heart. Being very small (weighing only 1·95 grm. in a lobster weighing 200 grm.), it was necessary to collect the hearts of several animals to make the analysis with any precision. The average of three determinations on different collections of material was 1·06 per cent., the lowest value being 0·85 per cent. The relatively high

percentage of glycogen in this primitive form of heart is interesting in view of the fact that a similar result was obtained in the heart of fishes. Further reference to the significance of this observation will be found in connexion with the latter.

With regard to the liver, the percentage amounts of glycogen varied considerably (viz. between 0·05 and 0·78 per cent.), thus contrasting with the muscles, where the amounts were tolerably constant. No doubt, as in mammals, the glycogen content of this viscus depends primarily on the activity of digestion, which it is presumed must have varied for different individuals. The largest result was obtained in a specimen caught in September.

## IV. THE FISHES.

Table IV.—Percentage of Glycogen in Various Parts of the Body of Fishes caught during July and August.

| No. | Species. | Organ or tissue. | Weight of material (grm.). | Glycogen percentages | |
|---|---|---|---|---|---|
| | | | | Calculated from original material. | Calculated from alcohol-preserved material. |
| H | Elasmobranchii (Squalus Sucklii), dog-fish | Liver | 44·2 | 0·057 | 0·069 |
| I | ,, | ,, | 20 | 0·16 | 0·209 |
| V | ,, | ,,* | 19·8 | None | None |
| K | | Muscle (body wall) | 50 | 0·018 | 0·025 |
| W | | Muscle* | 19·8 | None | None |
| G | | Heart | 7·3 | 0·447 | 0·847 |
| U | | ,,* | 4·6 | ... | 0·172 |
| Z | Chimæra (rat-fish) . . | Liver† | 16·7 | None | |
| $A_1$ | | | 15·2 | ,, | |
| | Teleostomi (Cyprinus carpio), carp | Muscle | 10·3 | ,, | |
| p. 95 | | (a) Liver | 3·44 | (a) Trace | |
| 96 | | (a) Muscle | 50 | (a) ,, | |
| 99 | | (b) ,, | 50 | (b) 0·021 | (b) 0·028 |
| 23 | | Liver | 10 | 6·50 | |
| 24 | | Muscle | 20 | 0·29 | |
| 25 | | Liver | 10 | 5·60 | |
| 26 | | Muscle‡ | 20 | Trace | |
| 98 | Christivomer Namaycush (lake trout) | (a) Liver | 26·6 | None | |
| 98 | | (b) ,, | 45 | 0·055 | |
| 97 | | (a) Muscle | 13·91 | Trace | |
| 97 | | (b) ,, | 11·70 | ,, | |

* Dog-fish caught in nets and kept in small tank for some days. Otherwise the dog-fish were freshly caught by line from the end of the wharf.

† Dead some time.

‡ Fish dead at least 24 hours.

The outstanding feature of most of these results is the absence or the small amount of glycogen. The organs were removed for chemical investigation immediately after the fish were caught; except in the case of certain of the dog-fish and the rat-fish, in which the fish, entrapped in nets, were brought to the station in a half-dead condition in the tank of the boat. The results stand out in sharp contrast with those of Schöndorff and Wachholder already referred to, in which the liver and muscles of a long list of fresh-water (Rhine) fishes show in general as high percentages of glycogen as have been found in any animal. The only fundamental difference between the two series of observations is with regard to the time of year at which the fish were caught. The difference cannot at all depend on whether the fish was caught in fresh or in sea water, since in our series fish from both were examined. The carp numbered 21–26 inclusive were obtained in November from a local dealer. Two were alive when brought to the laboratory. It will be noted that decidedly more glycogen was found than in the fishes examined in August (Nos. 95–99). Although not conclusive, this supports the view that the glycogen content of fishes is very low in the summer months and high in winter.

The very low glycogen content of fish caught in summer, as compared with the decidedly high percentage in winter, is in conformity with similar observations in Amphibia and hibernating mammals. Thus Athanasiu (op. cit.) found in the liver of Tusca 2·77 per cent. and 4·35 per cent. in July, and 8·21 per cent. and 7·52 per cent. in October and November. Lesser (20) and Bleibtreu (21) have also demonstrated a great decrease of glycogen in the liver and other tissues of frogs during the spring and summer months (accompanied by a steady increase in the ovaries) and its great abundance in the late autumn and early winter.

One other fact deserves attention in connexion with these results, namely, the relatively high percentage of glycogen in the heart of the dog-fish. In hearts from two recently caught dog-fish 0·447 per cent. (of fresh weight) of glycogen was found, when the livers of the same fishes contained only 0·057 per cent. and 0·16 per cent., and the muscles of the body wall only 0·018 per cent. In hearts from other dog-fishes that had been caught for some time and were partially asphyxiated, 0·172 per cent. (of alcohol-dried tissue) glycogen was found when no trace could be detected in the liver or muscles.

The presence of a large amount of glycogen in heart muscle has been known for some time (cf. Pflüger, loc. cit.), and has recently been confirmed by Cruickshank (23), who found in the heart of the dog from 0·300–0·631 per cent. It has further been shown by histo-chemical methods that the conducting tissues (the A–V node and A–V bundle) are especially rich in glycogen, a fact which is of particular interest in the light of our observation that there is a very high percentage in the primitive heart of the dog-fish, and also in that of the lobster. We were unfortunate

in failing to secure more material upon which to follow up this interesting observation.

## Conclusions.

By Pflüger's method the following percentage amounts of glycogen were found present in the digestive gland (hepato-pancreas) of representative species from various aquatic phyla:—Asteroidea, 0·232 to 1·52; Lamellibranchiata, 0·31 to 1·56; Crustacea, 0·05 to 1·39; Elasmobranchii, none to 0·21; Teleostomi, none to 6·5.

The varying amounts are apparently dependent partly on feeding conditions and partly on the season.

In the muscles the following percentage amounts were found:—Lamellibranchiata, 0·077 to 2·67 (the latter value was obtained in the adductor muscles); Crustacea, trace to 0·36; Elasmobranchii, none to 0·018; Teleostomi, none to 0·29.

In all cases where it was possible to secure a sufficient amount of heart muscle the glycogen content was found to be several times greater than that of the other muscles, and sometimes greater than that of the liver; thus in the lobster from 0·85 to 1·42 per cent. was found in the heart, compared with a maximum of 0·36 per cent. in the muscles.

In several cases the glycogen was found to yield a yeast-fermentable sugar after hydrolysis, but in others there is some evidence that a certain proportion of the "reducing material" was due to other substances.

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UNIVERSITY OF TORONTO STUDIES

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*Studies on the Respiratory Centre*

# I. THE BEHAVIOUR OF THE RESPIRATIONS AFTER DECEREBRATION IN THE CAT

By J. J. R. MACLEOD, M.B., F.R.S.C.

(Read May Meeting, 1919.)

Our knowledge of the functions of the respiratory centre depends on observations which have been made either on anæsthetised laboratory animals or on man in the normal state. To both groups of researches serious objections can be raised; to the former because of the use of anæsthesia, which is well known greatly to depress the excitability of the respiratory centre, and to the latter because of the limited variety of observations which it is practicable to make. As a first step to a further investigation of the respiratory function, therefore, it became necessary to seek for some method applicable to laboratory animals, in which the activities of the centre would not be dulled by the use of anæsthetics. It has been found that this requirement can be satisfactorily met by using the decerebrate preparation which was originally described by C. S. Sherrington,[1] and subsequently more closely studied by Theile,[2] Sherrington,[3] Forbes and Sherrington,[4] Miller and Sherrington[5], Weed[6] and Cobb, Bailey and Holtz[7].

The section of the brain stem is usually made about the level of the anterior corpora quadrigemina. After the effects of the initial anæsthesia have passed off a state of rigidity (plastic tonus) of the postural musculature supervenes and the breathing usually remains more or less normal.

In using the decerebrate preparation for investigation of the respiratory function one must not lose sight of the fact that important controlling influences have been removed from the centre, namely those derived from the higher cerebral centres, and that the respiratory function under these conditions may be as far removed from the

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normal as in an anæsthetised animal with brain intact. It is clear, however, that important facts are likely to be revealed by observing the behaviour of the decerebrated animal in relationship to changes in the chemical condition of the arterial blood, an investigation which is very difficult in man, and which can be carried out in anæsthetised animals only to a limited degree because of the presence of anæsthetics in the blood.

It has been noted by several of the above-mentioned workers, particularly by Theile (loc. cit.) and Weed (Weed, L. H., Am. Journ. Physiol., 1917, XLIII, 131.) that the character of the respirations does not remain the same throughout the period during which the animal survives the decerebration. Weed recognizes three periods of somewhat different behaviour of the decerebrated animals; in the third of these, which supervenes in from two and one-half to three hours following the decerebration, increasing respiratory difficulties with rapid decline are often observed.

In a series of investigations, conducted in the author's laboratory by R. W. Scott, in which the particular problem was to study the influence of rapidly increasing percentages of carbon dioxide in the inspired air and of intravenous injections of alkali on the respiration of decerebrate cats (Scott, R. W., Am. Journ. Physiol., 1917, Vol. 44, 196), it was noted that there were three more or less distinct groups of animals. In one of these, adequate spontaneous respiration did not return, or if it did so it was irregular and incapable to maintain life. In a second group the breathing was fairly satisfactory for the first hour or so but then became dyspnoeic and gasping, and the animal soon succumbed. In the third group the animal breathed with perfect regularity for many hours, and even at the end did not develop the dyspnœa characteristic of the second group.

Since these variations might lead to serious confusion in the further researches which were contemplated, it was decided to make a closer study of the breathing and particularly to see whether variations in it are correlated with changes in the acid-base equilibrium of the blood. It is with this phase of the problem that the present paper is concerned.

## Methods

Decerebration was performed with the apparatus and by the method described by Miller and Sherrington (Quart. Journ. Physiol., 1915, IX, 107). It is of interest to note that frequently the respirations became regular almost immediately after the decerebration and

remained so, particularly in young animals, even during compression of the vertebral arteries; whilst in other animals apnœa gradually supervened, but could be immediately terminated by allowing some arterial blood to reach the medulla by momentarily releasing the vertebrals. The reflex activities of the decerebrate animal varied considerably according to the exact position of the cut. When this was well forward of the anterior corpora quadrigemina the animals, after the effects of the anæsthetic had passed off, were highly excitable and behaved as if they felt some pain. Section by a scalpel a little further back immediately removed these mimetic reactions, but the resulting preparation was not as a rule so satisfactory as when the first cut had been in the correct position (*i.e.* through the anterior corpora quadrigemina). When the cut involved the posterior corpora quadrigemina spontaneous breathing rarely returned except in very young animals, and even in them it was usually irregular and unsatisfactory. In a succeeding paper we shall show that an abundant oxygen supply to the centre could usually be counted on to restore the breathing to normal in those cases in which it was irregular and spasmodic.

The alveolar air was collected by inserting into the trachea (through a side tube in the tracheal cannula) a narrow tube (gum-elastic catheter), the outer end of which was connected with an all-glass graduated syringe (10 cc., but capable of holding about 16 cc.) with the piston well smeared with vaseline. Towards the end of normal respiration the piston was withdrawn taking in from one half to one cubic centimetre. This process was repeated for several succeeding expirations after which the air was expelled again into the trachea so as to wash out the dead space of the tubing. By a repetition of the above procedure a sample of about 10 cc. of alveolar air was then collected for analysis. The analysis was carried out in a 10 cc. Haldane gas burette, the accuracy of which had been carefully checked against Brodie's apparatus.

There can be no doubt that the fractions of air collected towards the end of normal expirations is alveolar air in the usually accepted sense. The average tidal air of a cat is generally about 35 cc. and the dead space from the point of insertion of the catheter to the alveoli cannot be more than 10–12 cc. so that the last few cubic centimetres of air of a normal expiration, from which the fractions of 0·5–1 cc. are collected, must be undiluted alveolar air, even after allowing for the possibility that some of the peripheral layers of air in the trachea do not move so quickly as the axial currents (cf. Henderson, Chillingworth and Whitney, Am. Journ. Physiol., XXXVIII, p. 1).

Short of making actual comparisons of the tension of carbon dioxide in arterial blood and alveolar air, the most satisfactory evidence that the percentage composition of the alveolar samples collected by the present method really corresponds to the tension of the gas in arterial blood is supplied by the constancy of the results in successive samples of air. This constancy can be seen in the figures given in the tables accompanying this paper.

The percentage of carbon dioxide in the arterial blood was determined by the method of Barcroft and Haldane, using 0·5 cc. of blood and an excess of weak ammonia water, so that the precipitate which is formed when acid is added to dislodge the $CO_2$ does not make the solution so thick as to interfere with the evolution of the $CO_2$. The $P_h$ of the arterial flow was measured by the method of Levy, Rowntree and Marriott (Archiv. of Int. Med., 1915, XVI, 389), the exact $P_h$ of the phosphate solutions used for comparison being determined by the electro-metric method.

The total acidity of the urine was computed by adding (1) the titratable acidity (in cc. $n/_{10}$ acid per 100 cc. urine), after shaking with excess of neutral potassium oxalate, using phenolphthalein as indicator, and (2) the ammonia, using the permutit method of Folin and Bell (J. Biol. Chem. N.Y., 1917, XXIX, 329).

The quantity of lactic acid in the blood was determined by the modified von Fürth method described elsewhere by the author (Macleod and Hoover, Am. Journ. Physiol., 1916–17, XLII, 460).

## Consideration of Results

The present communication concerns the results obtained on cats which continued to breathe more or less normally for at least two hours following the decerebration. According to the behaviour of the breathing, as judged from the minute-volume of respired air, these animals could be divided into two main groups; in the one, the breathing either remained about constant or it slightly decreased, whilst in the other it progressively increased. In several animals of the first group the observation was terminated by bleeding to death, but in those of the second group it was usually continued until the animal died.

The results of a typical experiment of the first group of animals are given in Table I:

TABLE I

| No. Expt. | Time after decere-bration (min.) | Respirations per min. | | Alveolar $CO_2$ % | Blood $CO_2$ % | Acid of Urine c.c.n/10 % | Rectal temper-ature | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | Vol. c.c. | rate | | | | | |
| XVIII | 15 | .. | .. | .. | .. | 168 | .. | |
| | 45 | 770 | .. | 3·6 | .. | .. | 37·5 | |
| | 60 | .. | 38 | 3·75 | .. | .. | 38 | Rigidity |
| | 67 | .. | .. | .. | 33 | .. | .. | moderate acoustic reflex and hyperexcit-able. |
| | 75 | .. | .. | 3·70 | .. | .. | .. | |
| | 92 | .. | .. | .. | 39·6 | .. | .. | |
| | 95 | 645 | .. | .. | .. | .. | 38·4 | |
| | 113 | .. | .. | 3·2 | .. | .. | .. | |
| | 125 | .. | .. | .. | 40·9 | .. | .. | |
| | 127 | 600 | .. | .. | .. | .. | .. | |
| | 142 | .. | 30 | .. | .. | .. | .. | |
| | 160 | .. | .. | 3·1 | .. | .. | .. | |
| | 175 | 570 | .. | .. | .. | 86 | .. | Glycosuria. |
| | 200 | .. | .. | 3·0–3·5 | .. | .. | 37·3 | After warm-ing tank. |
| | 225 | 616 | .. | .. | 38·2 | .. | .. | |
| | 238 | .. | 33 | .. | .. | .. | 38·5 | |
| | 257 | .. | .. | 3·2 | .. | .. | .. | Rigidity less. |
| | 275 | .. | 30 | .. | .. | .. | .. | |
| | 285 | .. | .. | (3·6) | .. | .. | .. | Rigidity almost gone. |
| | 316 | .. | .. | 3·2 | .. | .. | .. | |
| | 330 | .. | .. | .. | 42·8 | .. | .. | |
| | 333 | 662 | 39 | .. | .. | .. | | |
| | 338 | .. | .. | .. | .. | .. | .. | B.P. 70 mm. Hg. |
| | | | | | | 90 bled to | death | Glycosuria. $P_h$ of blood at end 7·6 |

In more condensed form the results of the other observations of this group are given in Table II:

TABLE II

| No. Expt. | Time after decere-bration (min.) | Respirations per min. | | Alveolar $CO_2$ % | Blood $CO_2$ % | Urine Acid c.c. N/10 % | $NH_2$ c.c.N/10 % | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | Vol. c.c. | rate | | | | | |
| VI | 50-72 | .. | 60 | 3·3 | 44·2 | 120 | .. | $P_h$ of blood 7·4 |
| | 157-165 | .. | 32 | 2·75 | 41 | .. | .. | Decerebrate rigidity distinct. |
| | 190-195 | .. | 40 | 2·25 | 41 | .. | .. | Temp. 39°C. $P_h$ of blood 7·4. Bled to death |
| XIV | 60-100 | 1100 | .. | 3·5 | 52·8 | .. | .. | Marked rigidity |
| ♀ | 135-165 | 980 | .. | 2·8 | 38·5 | .. | .. | Rect. temp: 40°C. |
| | 190-225 | 855 | .. | 2·7 | 37 | .. | .. | Rigidity pronounced. |
| | 270-285 | 875 | .. | 2·9 | 38·2 | .. | .. | Rect. temp. 39°C. |
| | 290-310 | 760 | .. | 2·9 | 40·2 | .. | .. | Lactic Acid 0·175% |
| XXVI | 65-125 | 1000 -1040 | 40 | 4·5 | .. | .. | .. | Rect. temp. 39°C. |
| | 155-215 | 825 | 32 | 4·1 | .. | 28 | .. | Moderate rigidity |
| | 230-240 | 845 | 34 | 4·4 | .. | .. | .. | Lactic acid 0·081% |
| | 260-275 | .. | .. | 4·4 | .. | 16 | .. | No glycosuria |
| XXIII | 135-140 | 1080 | 27 | 3·3 | .. | 106 | 0·107 0·076 | |
| ♀ | 195-215 | 1120 -1170 | 28 | 3·3 | .. | .. | .. | Rigidity slight |
| | 230 | 1150 | 30 | .. | .. | 20 | 0·033 | |
| | 250-255 | 1120 | 28 | 3·0 | .. | .. | .. | |
| | 290-295 | 940 | 25 | 2·9 | .. | .. | .. | $P_h$ of blood 7·6–7·7 |
| | 302-305 | 960 | 22 | .. | 45 | 6·5 | | Lactic acid 0·098% 0·101% |
| XII | 45-60 | .. | 64 | 3·6 | 40·2 | .. | .. | Rigidity moderate |
| ♀ | 110-115 | .. | 42 | 3·9 | 39·6 | .. | 38·5 | |

TABLE II—*continued*

| No. Expt. | Time aster decere-bration (min.) | Respirations per min. Vol. c.c. | Respirations per min. rate | Alveolar $CO_2$ % | Blood $CO_2$% | Urine Acid c.c.N/10 % | $NH_2$ c.c.N/10 % | Remarks |
|---|---|---|---|---|---|---|---|---|
| | 135-147 | .. | 36 | 3·9 | .. | .. | .. | Rigidity moderate |
| | 217-247 | .. | .. | 3·1 | 41·0 | .. | .. | |
| | 265-285 | .. | .. | 2·4 | 37·0 | .. | .. | |
| | 305 | 412 | 42 | 2·0 | 38·0 | .. | 39·5 | B.P. very low |
| IX | 60-79 | 217 | .. | 4·3 | 40·8 | .. | .. | Respiratory Quotient 0·68 |
| | 98-126 | 294 | 14 | 4·8 | .. | .. | .. | Rigidity marked |
| | 131-146 | 394 | 16 | 4·5 | 44 | .. | .. | Respiratory Quotient 0·74. Rigidity slight. Lactic acid 0·01 % |

The following are considered the most noteworthy characteristics of these observations:

1. Both the respiratory volume and the respiratory rate decline gradually, although occasionally after four or five hours a slight increase may occur (cf. IX and XVIII). This increase is doubtless explained by the fact that the respiratory centre is hyperexcitable in decerebrate animals, so that the manipulation involved in withdrawing blood from the femoral artery for analysis, or in cleaning the femoral cannula of clots brings on a marked hyperpnœa which may last for some minutes after the irritation is removed.

2. The percentage of carbon dioxide in the alveolar air collected in from one to two hours after decerebration (when all ether has disappeared from the blood) varies between 3·3 and 3·9 in five of the six cats of this group, and in numerous observations which we have subsequently made this value has usually been found. Occasionally, and for no evident reason, the percentage may be somewhat higher as in experiments IX and XXVI. During the remainder of the period of observation the percentage of alveolar $CO_2$ either remains practically unchanged (XXVI) or it very gradually decreases.

In a series of decerebrate cats used in a research by Lois Fraser, R. S. Lang and the author, the alveolar air was analysed for both carbon dioxide and oxygen, and it is of importance in the present connection to place on record the respiratory quotients obtained in samples of air removed at varying periods over a time in which the animal was breathing normally. Table III gives these results.

TABLE III

| No. of Cat. (new series) | Time after decerebration (minutes) | Respiratory Quotient |
|---|---|---|
| X | 100 | 0·71 |
| | 175 | 0·72 |
| | 210 | 0·71 |
| XX | 80 | 0·92 |
| | 115 | 0·90 |
| | 131 | 1·04 |
| XXIII | 70 | 0·72 |
| | 90 | 0·70 |
| XXIV | 60 | 0·89 |
| | 86 | 0·77 |
| | 172 | 0·81 |
| | 183 | 0·82 |
| XXV | 68 | 0·72 |
| | 83 | 0·71 |
| | 100 | 0·68 |
| XXVI | 126 | 0·81 |
| | 140 | 0·72 |
| | 161 | 0·85 |

It will be observed that, with two exceptions, the respiratory quotient varies in different animals from 0·7 to 0·9, indicating that a normal type of metabolism is in progress. The relative steadiness of the quotient in each animal further shows that the alveoli are being ventilated at a uniform rate.

3. The percentage of carbon dioxide in the arterial blood varies between 37 and 45 volumes per cent, with the exception of the first observations made on cats XVIII and XIV, in the former of which it is abnormally low and in the latter abnormally high. Both of these

exceptional results were obtained in blood removed in about one hour after decerebration, and subsequent experience has taught us that a longer period than this should have been allowed for the ether to have been expelled from the body. Throughout the remainder of the observations the carbonate of the arterial blood remains practically steady. It is impossible to say from the few results on hand and the small degree of fluctuation in the blood-carbonate values whether any parallelism exists between them and those of the aveolar $CO_2$.

4. Determination of the total acidity and ammonia content of the urine has not supplied results that can be satisfactory interpreted. In practically all cases, of this group as well as of others, in which sufficient urine was obtainable to estimate both the acidity and the ammonia content, a direct proportionality has been observed between the two, so that to follow changes in the acid excretion in a given cat either the titration or the ammonia values may be used. In the three experiments of this group in which there are adequate data a very decided decline in the acid concentration is observed, in fact in two of the animals, XXVI and XXIII, the urine became nearly neutral. Whether this result depends upon a failure of the kidney to remove acid radicles from the blood or upon a relative increase in fixed alkali in the organism cannot be said.

5. The hydrogen-ion concentration of the arterial blood remained normal, at $P_h$ 7·4, in one of the animals, but it became less, *i.e.* $P_h$ became greater (7·6–7·7) in two of them (XVIII and XXIII). On account of technical difficulties it was impossible to secure sufficient data to make certain that these changes are real, but further observations will be published shortly. If a real increase in $P_h$ does occur it would indicate that the decreasing acidity of the urine, above referred to, is dependent upon alkali retention.

6. Lactic acid was determined in the arterial blood of three of the experiments. In two of them (XXVI and XXIII) it varied between 0·081 and 0·101 per cent. In the third (XIV) it was much higher, namely, 0·175 per cent. It is important to note that the last estimation is possibly too high because the extraction with ether had to be performed in two portions of the unevaporated protein-free filtrate. This would relatively increase the error due to any impurities in the reagents.

It is of interest in this connection to place on record results obtained for lactic acid in the blood of two cats which were bled solely for this purpose immediately after anæsthetising with ether. These are as follows: 0·052 per cent (Cat No. XXVIII) and 0·113 per cent (Cat No. XXIX). It is clear that considerable variation exists in

the lactic acid content of cat's blood even under approximately normal conditions. Partial asphyxiation, due to the ether, may explain the variability.

7. Other occasional observations included testing the urine for sugar, the extent of muscular rigidity, and the arterial blood pressure. Glycosuria, when tested for, did not appear so frequently in the cats of this group as in those of the second group. On account of difficulties with clotting, to which the blood of many decerebrate animals appears to be very prone, and because of lack of assistance, it was impracticable to secure many records of arterial blood pressure. In about five hours after decerebration in Cat No. XVIII, however, it was 70 mm. Hg., and from the ease with which the blood flowed from the femoral artery for the lactic acid estimation, it must have been at this height, at least, in the case of the other experiments.

The degree of decerebrate rigidity varied considerably in the different preparations.

A typical observation of the group of animals in which hyperpnœa developed is given in Table IV:

In all of the animals of this group the decerebration was performed well forward of the anterior corpora quadrigemina and the decerebrated animal was very excitable, hyperpnœa being induced by the slightest disturbance. As a rule this hyperpnœa was transient (cf. Exp. XXII) and in all the animals the respirations progressively increased in volume and rate without any evident afferent stimulation. In the most extreme cases (Nos. X, XXX and XXXI) death occurred in about two hours after decerebration, being usually preceded by vomiting movements and convulsions. The rapid development of these conditions made it impossible to analyse many samples of alveolar air or blood for $CO_2$, so that attention was rather given to securing samples of blood and urine of adequate size so that lactic acid, H-ion concentration and the acid excretion might be ascertained. The following observations are noteworthy:

1. If we take the average minute volume of respired air of a normal decerebrate cat as 1000 cc. (cf. R. W. Scott, Am. Journ. Physiol., 1917, XLIII, p. 169), it is seen that in about one hour after decerebration all the animals of this group were respiring normally, although usually somewhat rapidly, the average for normal animals being 20–25 per minute. The hyperpnœa which subsequently developed either did so gradually (Nos. XVII and XXII) or, after doing so for a time, suddenly became much more pronounced (XXX and XXXI), this type being especially prominent in hyperexcitable preparations.

TABLE IV

| No. | Time after decerebration. min. | Respirations. cc. per min. | Alveolar $CO_2$ % | Blood $CO_2$ % | Urine n/10 acid % | n/10 $NH_3$ % | Rectal Temp. | Remarks |
|---|---|---|---|---|---|---|---|---|
| XVII | .. | .. | .. | .. | 82 | .. | .. | Cut far forward. Perhaps slight asphyxia |
| | 25 | .. | .. | .. | .. | .. | .. | |
| | 45 | .. | 5·0 | .. | .. | .. | .. | B.P. 120 mm. Hg. |
| | 55 | .. | 4·9 | .. | .. | .. | 39 | |
| | 60 | .. | .. | 41·6 | .. | .. | .. | Rigidity slight |
| | 75 | 916 | .. | .. | .. | .. | 39·8 | B.P. 110 mm. Hg. |
| | 80 | .. | 4·5 | 43·6 | .. | .. | .. | |
| | 95 | 1020 | .. | .. | 88 | 0·16 | .. | Glycosuria |
| | 105 | .. | .. | .. | .. | .. | .. | Occasional sighs |
| | 115 | .. | 3·4 | .. | .. | .. | 39·8 | |
| | 121 | 1130 | 3·1 | .. | .. | .. | .. | |
| | 134 | 1180 | .. | .. | .. | .. | .. | |
| | 150 | .. | 2·8 | .. | .. | .. | .. | |
| | 155 | .. | 1·8 | .. | .. | .. | .. | |
| | 160 | .. | 1·8 | .. | .. | .. | .. | B.P. 55–60 mm. Hg. |
| | 166 | 1430 | .. | 31 | .. | .. | .. | Rigidity slight |
| | 173 | .. | 2·0 | .. | .. | .. | .. | |
| | 176 | 1430 | 1·9 | .. | .. | .. | .. | |
| | 191 | 1720 | .. | 29 | .. | .. | .. | |
| | 205 | .. | 1·6 | .. | .. | .. | .. | |
| | 209 | 1500 | .. | 27·7 | .. | .. | .. | |
| | 215 | 1560 | .. | .. | .. | .. | .. | Very rigid. |
| | 235 | .. | .. | .. | 28 | 0·04 | .. | Glycosuria. Lactic acid 0·121 % $P_h$ not done |

The abridged results on other animals that showed the same behaviour are given in Table V:

in the lactic acid content of the blood, but it remains constant or increases in two experiments in which the hyperpnœa developed acutely (Nos. XXII and XXX) and an excess of lactic acid and a marked lowering in $P_h$ of the arterial blood is evident. Inasmuch as the acid excretion in all of the normal decerebrate animals becomes markedly depressed, it is significant that in those animals of the hyperpnœa group the acid excretion should have continued high.

7. Decerebrate rigidity was very marked in the four animals of this series in which there was excessive hyperpnœa and was slight in that (XVII) in which this was of lesser degree. This observation is possibly of interest, since it suggests that the accumulation of lactic acid, which was very high in two of these cases, may depend on the abnormally contracted musculature. According to such a view, in the state of permanent (plastic) tonus the blood supply to the muscles may be inadequate to supply sufficient oxygen to effect the oxidative removal of the lactic acid which therefore accumulates and overflows into the blood. With regard to the rectal temperature it will be noted that this usually rose somewhat, but not sufficiently to account for the dyspnœa.

Glycosuria of slight degree was observed in two of the experiments; sugar was not examined for in the urine of the other animals.

It may appear that the division of the animals into two groups, according to whether or not decided hyperpnœa became established, is arbitrary and that certain of the observations should have been classified as belonging to an intermediate group. Although this is true, it is nevertheless, we believe, more correct to adopt the present classification since it corresponds to the general impression, which is conveyed by actual experience with decerebrate animals. As a matter of fact, the above records include, out of a total of twenty animals, all those save one in which satisfactory spontaneous regular breathing existed one hour after the decerebration. In the one exception, the breathing was excessively rapid and deep, the aveolar $CO_2$ well below two per cent and the blood carbonate below twenty per cent. Although the rigidity was of slight degree this animal was extremely hyperexcitable, the section being well forward of the anterior corpora quadrigemina, and the hyperpnœa was definitely dependent upon afferent stimulation induced by faulty technique in catheterization.

In seven of the twenty animals satisfactory breathing did not spontaneously return within one hour after the decerebration, and the animals were discarded. It was usually the case that the section in their cases was well back, but its exact position has not been recorded.

We have preserved the anterior portions of the heads of numerous animals in subsequent experiments of a similar type, and we hope in the near future to be able to furnish data which will enable us to state precisely where the cut should be situated for satisfactory breathing. As far as we can say at present when the posterior corpora quadrigemina are even slightly wounded spontaneous respiration is seldom, if ever, observed.

In collaboration with Lois Fraser and R. S. Lang, I have found, however, that perfectly regular respirations may reappear in animals of the above type by greatly raising the partial pressure of oxygen in the alveolar air. This is done by passing a catheter into the trachea so that its open end lies above the bifurcation, and then discharging washed oxygen at a rapid rate from a cylinder of the gas. In a few minutes, during which artificial respiration may be necessary in order to carry the oxygen to the alveoli, the animal usually begins to breathe in perfectly normal fashion and continues doing so for hours. We are at present engaged in studying the very interesting and far-reaching problems which this observation presents; for the present we may point to the interesting evidence it affords that oxygen deficiency, *per se*, far from acting as a stimulus for the respiratory centre, renders it incapable of rhythmic function, at least in conditions where it is imperfectly supplied with blood, as after decerebration. In this connection attention should again be called to the fact that if the breathing of the decerebrate animal becomes feeble during compression of the vertebral arteries, it can be restored to normal by releasing the blood flow.

## Conclusions

After removal of the cerebral hemispheres a certain number of cats continue to breathe in perfectly normal fashion for several hours; others fail to respire adequately and still others breathe normally for some time, but subsequently become hyperpnœic, and finally are usually seized by convulsions to which they succumb. These differences in behaviour seem to be dependent upon the age of the animal and the level at which the section of the mesencephalon is made. Spontaneous breathing is decidedly more likely to return in the younger animals and when the cut is not further back than the anterior edge of the anterior corpora quadrigemina. When the cut is further forward the decerebrate animal is hyperexcitable, decerebrate rigidity is marked, and the animal usually becomes hyperpnœic. When the cut is farther back, adequate spontaneous breathing is unusual.

Particular attention is given in the present research to the possible cause of the hyperpnœa. With this object in view the behaviour of the percentage of carbon dioxide in the alveolar air and arterial blood as well as the hydrogen-ion concentration and the percentage of lactic acid in the latter have been compared with the respiratory behaviour of the animal. The acid excretion by way of the urine has also been observed.

It has been found that the above-mentioned values remain tolerably constant in the animals which do not become hyperpnœic, but that in those which do so the alveolar-$CO_2$ steadily declines, accompanied or followed by a decline in blood carbonates and by a decided increase in the hydrogen-ion concentration and lactic acid content of the arterial blood. These blood changes indicate that unoxidised acid, lactic, has accumulated in the blood and the main question to be considered is whether the hyperpnœa is the result of the accumulation of acid or whether the acid accumulates because of hyperpnœa. Concerning the first hypothesis the close attention which has been given in recent years to the condition known as acidosis has shown that there are three characteristic signs of the condition: first, a decrease in the percentage of carbon dioxide in the alveolar air; secondly, a decrease in the ability of the blood to combine with this gas, and thirdly, an increased excretion of free acid by the kidney. Now it will be noted that the first two of these characteristics are very prominently affected in those decerebrate cats which became hyperpnœic, and that the third—acid excretion in the urine—in the cases in which it could be measured, remained decidedly higher in the hyperpnœic animals than in those that breathed normally. If we add to these indirect evidences of an acidosis condition the further evidence afforded by a determination of the hydrogen-ion concentration of the arterial blood there seems little doubt that an intoxication by acid must have been the cause of the hyperpnœa. As to the nature and source of this acid there is evidence that it was lactic acid—large percentages being found in the arterial blood—which may have been derived from the plastic tonus of the muscles of the decerebrate animals, for this condition, though present in all the animals, was especially prominent in those that became hyperpnœic. It has been shown by Roaf (Quart. Journ. Exp. Physiol., 912, V, 31–53) that the gaseous metabolism of decerebrate cats is no greater than that of animals whose muscles are paralysed by curare, which indicates that very little energy can be expended notwithstanding the permanently contracted state of the muscles.

If we accept the modern view which is the outcome of the work of F. Gowland Hopkins and Fletcher (Proc. Roy. Soc. Lond., 1917, Ser.

B, LXXXIX, 444) that the lactic acid which is produced by a muscular contraction is removed by an oxidative process before the next contraction takes place, then it is conceivable that in the permanent contraction, to which plastic tonus corresponds, the acid fails to disappear from the muscle so that it overflows into the blood, in which it accumulates, since it can no longer be removed by oxidation, and from which it is only gradually excreted.

In brief, then, the simplest interpretation for the hyperpnœa and final collapse of many decerebrate animals is that it is caused by acute acidosis brought about by an accumulation of lactic acid derived from the permanently contracted extensor musculature which is characteristic of this condition. But we do not intend to imply that this hypothesis is proven by the observations of the present research. In so far as the results themselves are concerned there can be no doubt as to the reliability and tolerable accuracy of the values given for the carbon dioxide of the alveolar air and blood, but there is a possibility of error in connection with those for the $P_h$ and lactic acid content of blood. The greatest care has been taken in the analyses and they have always been carried out under strictly standardized conditions, but nevertheless it is possible that the samples of blood on which they were carried out were removed when the animal was already in a moribund condition, in which because of failing circulation such changes as were observed are to be expected. To circumvent this possible source of error blood was taken from one of the hyperpnœic animals (Table IV, No. XXX) while the arterial blood pressure was still above 70 mm. Hg., with the same results. Further observations of a similar type are, however, necessary.

With regard to the second hypothesis, namely that the organic acid of the blood rises to take the place of the carbonic acid which is blown off because of hyperpnœa induced by afferent stumuli acting on a hyperexcitable respiratory centre, little that is definite can be said. In support of this view, however, stands the fact that the animals exhibiting the hyperpnœa were invariably those in which, because of the forward position of the section, there was decided hyperexcitability of the nerve centres.

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# ON THE FORMATION OF HYDROCHLORIC ACID IN THE GASTRIC TUBULES OF THE VERTEBRATE STOMACH

BY

J. B. Collip, M.A., Ph.D.

# ON THE FORMATION OF HYDROCHLORIC ACID IN THE GASTRIC TUBULES OF THE VERTEBRATE STOMACH

## I. Literature

Since the time it was first recognized that hydrochloric acid was normally produced during digestion by the activity of the gastric mucosa, numerous attempts have been made to solve the physiological problem therein involved. The purely chemical aspect of the problem, the formation of a free mineral acid from an alkaline blood plasma, led many observers at different times to seek to discover the mode of formation and seat of origin of this acid, using for this object methods of investigation which we might term purely chemical. Others who were engaged in working on the histological structure of various parts, found, that, in the case of the stomach, their detailed morphological descriptions of the gastric mucosa afforded them ample material as a basis for lengthy speculations as to the correlations of the structures studied, and of the physiological processes known to be associated in one way or another with these structures. The literature, therefore, can be placed under two heads, the first dealing with the histological details and the speculations as to the function resulting therefrom; the second with the direct attempts to determine the place of origin of the acid itself. A summary of this literature under these heads, and in the order given, follows.

### 1. *The Morphological Data and the Discussions Thereon*

*The Mammalian Stomach.* The Mammalian stomach differs from that of all other Vertebrates in that in the gastric tubules of the fundus region two quite separate and distinct types of gland cells are manifested. These have now long been

known respectively as the chief or peptic cells and the parietal or border cells. This duality displayed in the structure of the Mammalian peptic glands or Labdrüsen was first described by Heidenhain (1870). He named the two forms which he had observed the "Hauptzellen" and the "Belegzellen" respectively.

Since that time much discussion as to the nature and significance of each of these two forms of gland cells has taken place. The writings in this connection have been very numerous, and the authors have used various qualifying terms in their descriptions of these cells. The chief cells or Hauptzellen have been spoken of also as "adelomorphe Zellen," "polygonale Zellen," "central cells," "celles principales," "Schleimzellen," etc. The parietal cells, or Belegzellen, have also been described as "delomorphe Zellen," "Pepsinzellen," "Kegelförmigezellen," "border cells" and "Cellule ricoprenti."

The great difference in size, in optical behaviour and in the staining capacities of the two types observed by Heidenhain led him to the conclusion that the chief cells had to do with the elaboration of the proteolytic ferment pepsin, and that the parietal cells supplied the greater part of the fluid of the gastric juice, and were also associated with the formation of the hydrochloric acid. Heidenhain also noted that the parietal cells were relatively more abundant in the upper third of the bodies of the gastric tubules of the fundus region. This, he thought, was very significant, as he had observed that extracts made from the deeper layers of the gastric mucosa had greater peptonizing power than similar extracts which had been prepared from the more superficial layers. Ebstein and Grützner (1872 and 1874) obtained similar results. They concluded that the greater part of the pepsin was probably elaborated by the chief cells. They advanced the view that the parietal cells were rich in the chlorides of the alkalies and by some unknown process liberated the free acid from these salts.

Nussbaum (1878) stated that the parietal cells stained more deeply with osmic acid one hour after the onset of activity than at any other time. He held to the view that the parie-

tal cells produced the pepsin, the chief cells along with the pyloric gland cells being mucin producers. He was also of the opinion that the parietal cells were homologous with the peptic gland cells of the lower Vertebrata, which, therefore, would produce pepsin.

Edinger (1879) held that a separate function for each type of cell could not be definitely proven.

Langley and Sewall (1879) and Langley (1881) studied the changes during functional rest and activity in the gastric tubules, and found that in the chief cells, when the glands were not secreting, were granules which began to disappear or to become fewer in number when secretion began. As the pepsin of the secretion or extractible from the mucosa either during rest or at any stage of activity was apparently in proportion to the number of granules present it was claimed that the granules themselves were composed of the mother substance (*zymogen*) of the ferment pepsin and that, therefore, the chief cells secreted pepsin. The parietal cells, on the other hand, were found free from granules during the resting stage and developed very fine ones during activity which, however, did not appear to be associated with the formation of any ferment. These cells were observed to become larger in size as the secretion began, and they remained enlarged as long as secretion continued. This made it evident that they secrete some constituent of the gastric juice. As the parietal cells are limited in their distribution to the cardiac portion of the stomach and as this portion alone furnishes an acid secretion the authors concluded that the parietal cells are concerned in the secretion of the hydrochloric acid of the gastric juice. Langley (1881) in consequence applied the term *oxyntic* (i.e., acid-forming) to these cells.

Greenwood (1885) found that the parietal cells stained quite markedly with nitrate of silver solution. This was probably the first direct evidence of their function.

Oppel (1896) maintained that a perfect functional division was impossible. There could only be a quantitative difference in the relative amounts of the constituents of the gastric juice

contributed by each type of cell. The parietal cells, he thought, were represented by the body cells of the fundus gland tubules of the lower Vertebrata, while the homologues of the chief cells were to be found in the neck cells of the gland tubules of these lower forms. Neither type of cell was a new departure in the Mammal, he claimed.

Bensley (1899) showed that in the fundus glands of the cat and the dog the chief cells were of two classes, those of the body and those of the neck of the gland tubule. The former were characterized by the possession of a large number of "zymogen" granules, which were distributed in varying extent throughout the luminal zone of each cell, and by a protoplasmic outer zone, distal from the lumen, which stained deeply with nuclear dyes, and which also presented a fibrillar appearance. These cells, he thought, were engaged in the secretion of a ferment. Those of the neck of the tubules did not contain, at any stage of digestion, zymogen in the form of granules, while their staining capacity indicated that they were engaged in the secretion of mucin. He held that the pyloric gland cells of the cat and the dog were analogous to this latter modification of the chief cells. The mucous neck cells of the glands of the fundus region of the frog's stomach together with the cells of the bodies of the pyloric glands were, he stated, the physiological and morphological homologues of this type, with which they corresponded in both position and functional changes as well as in staining properties.

From the foregoing it may be gathered that the structures of the chief cells of the body of the fundus gland tubules and the changes which they have been observed to undergo during their various phases of activity indicate their main function to be that of elaborating the pepsin of the gastric juice. The function of the parietal cells, however, presents a more difficult problem. Their structural detail will not permit of drawing any sweeping conclusion. As Heidenhain early pointed out (1870) they were very large in comparison with the chief cells, the cell body consisting of a very finely granulated protoplasm in marked contrast with the zones of coarsely granu-

lated protoplasm seen in the chief cells. The only change observed to take place during activity was in respect to their bulk. They are larger during activity. The cell protoplasm of the parietal cells stains very deeply with such dyes as eosin and acid fuchsin, and their differentiation is thus an exceedingly simple matter. They also occupy a position distal from the lumen, but with which they are nevertheless in direct communication by means of diverticula of the lumen extending between the chief cells. The bodies of the parietal cells themselves are permeated also by a system of fine intracellular canals which communicate with the diverticula. The fact that the parietal cells are restricted in their distribution to the glands of the cardiac portion of the stomach, taken in conjunction with the physiological evidence that the secretion of the pyloric glands is alkaline in reaction, seems to indicate that these cells are in some manner associated with the acidity of the secretion of the fundus glands. That they do not at all exercise a pepsin-secreting function cannot, however, be maintained on histological evidence.

The parietal cells are undoubtedly a unique type in the mammalian stomach. The question therefore arises: Are they entirely a new development in this, the highest order of the Vertebrata: or are they represented, or is their appearance foreshadowed, in any of the lower Vertebrates? The idea which has generally prevailed, as will be shown, has been that the parietal cells are represented in the lower forms of Vertebrates, and that they are simply a specialized and highly modified type in the Mammalia. Many authors, too, on such grounds, have ascribed the peptic function to the parietal cells.

*The Gastric Glands of Birds.* The stomach in birds consists essentially of two parts, the glandular stomach or "Drüsenmagen" and the muscular stomach or "Muskelmagen" with, in many cases, an intermediary zone between these two regions. The muscular stomach has a chitinous lining which is being constantly renewed by the secretion from the modified mucous glands. The glands producing the acid and the pepsin of the gastric juice are entirely confined to the "Drüsen-

magen" or "first stomach." This organ is manifested as a dilation of the oesophagus for a short distance before its junction with the gizzard or muscular stomach. The wall of this region is thickened, owing to the presence in it of the especially modified and very much aggregated gastric tubules in the form of compound glands. The opening of each of these compound glands into the central lumen is marked by a papilla. When the "first stomach" or proventriculus, as it is called, is laid open the wall is seen to be dotted with these papillae. The mucosa of the wall between the papillae is folded into short mucous crypts.

Réaumur (1752) noted the presence of the papillae, while Molin (1850) described quite accurately the finer structure of the proventriculus itself. Hasse (1866) stated that the secretion expressed from the compound glands was quite clear. Cazin (1888) divided the cells forming the compound glands into two classes: the peptic cells, which formed the peripheral tubules, and the mucous cells, which lined the wall of the collecting channels. Klug (1893) described the peptic cells of the compound gland tubules as being finely granular and having no restricting cell membrane. The nucleus he found to be more or less central in position, as is the case in the parietal cells. He held that these cells, which he found to be all of one type, secreted both the acid and the pepsin of the gastric juice, and that they were homologous with the parietal cells of the Mammalian gastric tubules.

Only one type of glandular cell has, therefore, been recognized in the peripheral gastric tubules of the compound glands of the bird's proventriculus. This type may be compared with the parietal cell of the Mammal, yet such a comparison can only be of the most general character. The cell body itself in its staining properties is more like the chief cell. But here again, there can be found no broad basis of comparison. It subserves the same functions as both the parietal and chief cells combined, and therefore one might expect that points of resemblance with both these types would be found, and indeed this seems to be the case.

The very slight amount of interstitial tissue which can be demonstrated between the compound gland tubules is noteworthy, and as the Mammalian stomach, large lymph nodules are of frequent occurrence on the walls of the secreting tubules.

*The Gastric Glands of Reptiles.* The stomach in the Reptilia is usually very much elongated, but the pyloric and fundus regions are always capable of definition, microscopically at least (Oppel 1896). The glands of the fundus vary considerably in size and structure. The crypts may be very deep and lined by the typical neck cells of the mucus-elaborating variety, or again, comparatively short but always showing the same type of neck cell. The body of the gland tubule is constituted of one type of secreting cell. The pylorus gland cells resemble the neck cells of the fundus region.

Partsch (1877) showed that both the pyloric region of the stomach and the oesophagus gave extracts with a very low pepsin content, while from the fundus region extracts of high digestive power could be made.

*The Gastric Glands of Amphibians.* The body of the "Labdrüsen" or peptic gland tubules of the Amphibia contain but one type cell (Oppel 1896). The neck cells of the fundus region and the pyloric gland cells afford practically the same comparison as their homologues in the Reptilia and Mammalia. In Batrachians, such as the frog (Langley, 1881), the peptic glands are not confined to the cardiac portion of the stomach for the oesophageal glands secrete pepsin, but not an acid juice.

*The Gastric Glands of Fishes.* The peptic glands of the order Pisces are formed of one type of cell only. The size of the cells and the nature and extent of their granulation vary in the different species. They are, generally speaking, of the same type as their homologues in the Amphibian or Reptilian stomach (Oppel, 1898).

Richet (1878) noted that the hydrochloric acid in gastric juice of certain fishes was represented by a concentration of from ten to fifteen grams per litre. The highest concentration

which has yet been observed in the Mammalian stomach was 0.58 per cent. (Rosemann, 1907).

This review of the histological structure of the gastric tubules of the lower Vertebrates suffices to show that in considering either the acid or the pepsin-elaborating function of these glands we shall, in all cases, with the exception of certain Batrachia, have to deal with one type of cell only. These cells have the same functions as are performed by the parietal and chief cells of the Mammals. An investigation which would reveal the manner in which the hydrochloric acid of the gastric juice of the lower Vertebrates is elaborated by the gland cells of the gastric tubules should, therefore, throw some light upon, and assist in the understanding of, the acid-forming function of the Mammalian stomach.

2. *The Data from the Application of Chemical Methods*

The microchemical method was first applied by Claude Bernard (1850) to determine the seat of origin of the hydrochloric acid. He injected into the jugular vein of a rabbit a solution of lactate of iron followed by one of potassium ferrocyanide. He hoped thus to detect the place of origin of the acid as no precipitation of the Prussian blue could occur in the circulatory system since the blood was alkaline, while such a precipitation would take place in any tissue or fluid of the body which had an acid reaction when the two salts reached that tissue or fluid. After three-quarters of an hour the animal was killed and the stomach immediately laid open. A deposit of Prussian blue was found on the surface of the mucosa, which was especially marked in the region of the lesser curvature. Microscopic examination, however, revealed no blue other than on the surface of the mucosa. Bernard concluded, therefore, that the acidity of the gastric juice was only manifested after the secretion from the tubules had reached the surface and had become mixed with the fluids of the stomach.

Brücke (1859) was of the opinion that the hydrochloric acid was produced free in the gastric tubules. He pressed freshly cut sections of the proventriculus of a pigeon, actively

digesting, between litmus paper. The free surface of the mucosa proved to be strongly acid in reaction while the multilobular glands imparted a neutral or only a very slightly acid tint to the litmus. The mucosa of a rabbit's stomach was likewise found to have an acid reaction on the surface but to be neutral in the deeper portions. Brücke assumed that, if a free acid was liberated in the gastric tubules, an alkaline fluid of corresponding strength must at the same time pass into the blood and lymph systems. Thus he explained the non-occurrence of an acid reaction in the tubules themselves.

Lepine (1872) repeated Bernard's experiment and obtained similar results. He tried also other methods in attacking the problem. He macerated in a solution of lactate of iron the gastric mucosa of an animal which had previously been injected with a solution of potassium ferrocyanide. Negative results only were obtained by this method. While he was not able to show that either type of cell was acid in reaction, he nevertheless held that his failure did not entirely preclude the possibility that one variety of cell had the function of preparing the acid if not of completely elaborating it.

Edinger (1882) introduced several new methods. He used tropaeolin and phenolphthalein, but with these he obtained no definite results. Noteworthy findings were obtained when concentrated neutral solutions of sodium alizarin were employed. Neutral solutions of sodium alizarin are purple red in colour. The addition of a drop of acid causes the precipitation of the yellow colloidal alizarin. He found that the gastric mucosa of rabbits and dogs was flecked with small areas which had a yellow colour after he had injected into their blood circulation a concentrated neutral solution of this substance. These yellow patches were separated from one another by broad red violet zones. The yellow colour could, in sections, be traced into the deeper part of the tissue, but no positive information as to its precise locality was obtained owing to the faintness of the colour in thin sections. This reaction did not obtain in the mucosa of fasting dogs. The occurrence of the isolated yellow patches in the wall of the mucosa of the supposedly

active stomach, Edinger explained as due to only small areas of the mucosa being in full secretary activity at any one time.

Trinkler (1884) also used tropaeolin with negative results. He concluded that neither chief nor parietal cells formed free hydrochloric acid.

Stintzing (1889), using Congo red, which in the presence of a mineral acid changes from deep red to blue, obtained in some parietal cells of the fundus glands of various animals blue granules of varying sizes. This he held as evidence supporting the view that hydrochloric acid was elaborated by the parietal cells.

Sehrwald (1889) utilized the Prussian blue method in a modified form. The fresh gastric mucosa was placed for a day in a solution of lactate of iron, then washed and transferred to a solution of potassium ferrocyanide where it remained for some time. After this treatment the parietal cells were found to stand out clearly, owing to a large content of Prussian blue.

Fränkel (1891) showed, however, that results similar to those of Sehrwald's could be obtained when alcohol-hardened tissue was used instead of fresh material. He also repeated Edinger's sodium alizarin experiment and confirmed his findings. He pointed out that this method was not without objection, however, as the reaction could be brought about by the acid resulting from the dissociation of the neutral salt. Fränkel injected intravenously decolorized solutions of acid fuchsin. In the active stomach of the dog he found the whole surface of the mucosa stained a brilliant red. Teased out tissue showed both the chief and parietal cells of the fundus gland tubules to be coloured intensely red. The cylindrical epithelium and the interstitial tissue were not coloured. He only obtained the colouring in patches on the surface of the mucosa of the rabbit. He considered that his results proved that the gastric tubules were acid in reaction, but did not think that the formation of the acid could be ascribed to any one type of cell.

Gmelin (1902) held that an inter-relationship between the

parietal cells and the formation of the free hydrochloric acid was not yet proven.

Miss M. P. FitzGerald (1910) undertook, under Professor Macallum's direction, to determine if the Prussian blue reaction could not still be applied to reveal successfully the seat of origin of the hydrochloric acid of the gastric juice. She employed potassium ferrocyanide and the double citrate of iron and ammonia which was substituted for the lactate of iron used by earlier workers. She found that weak neutral solulutions of potassium ferrocyanide and the citrate of iron and ammonia could be mixed and allowed to stand without a trace of Prussian blue being precipitated. The addition thereto of sodium dihydrogen phosphate, sodium hydrogen carbonate and carbon dioxide did not cause a precipitation of the Prussian blue to take place. The slightest trace of hydrochloric acid, however, brought about an immediate precipitation. The double citrate of iron and ammonia was found to be neutral in reaction, and in this respect wholly unlike the lactate and other iron salts previously used. Both Lepine (1872) and Sehrwald (1889) had observed the acid reaction of iron salts in solution, and the spontaneous formation of Prussian blue when a solution of ferric lactate is mixed with one of potassium ferrocyanide. A balanced mixture of sodium or potassium ferrocyanide (preferably sodium ferrocyanide, as it is less toxic than the potassium compound) and the double citrate of iron and ammonia, makes, therefore, an excellent reagent for the detection, by microchemical means, of minute quantities of acid in the body.

Miss FitzGerald, working with rabbits, dogs and guinea pigs, obtained a series of varied results by this method. After intravenous injections of quite small amounts of solution formed of equal volumes of an aqueous solution of 2.25 per cent. of ammonium ferric citrate, and of a 1.5 per cent. potassium ferrocyanide, there occurred in nearly all cases a deposit of Prussian blue on the surface of the gastric mucosa, which was usually limited to the region of the lesser curvature. In a few instances the deposit of Prussian blue was not confined

to the surface of the mucosa, but was found to be present also in the crypts and, at isolated points, in the upper two-thirds of the lumina of some of the gland tubules. In examination of such preparations with the high power objectives the canaliculi of the parietal cells and their connections with the gland lumen stood out clearly, in consequence of the deposit of Prussian blue within them.

Apart from the positions just mentioned, Prussian blue was also found in some adjacent lymph vessels, a few blood vessels, and in certain wandering cells and leucocytes between the gland tubules. In no other situation was a deposit of Prussian blue observed, but the immersion of portions of the various tissues in dilute hydrochloric acid caused the blue precipitate to be formed, showing that both salts essential for the reaction were quite uniformly distributed.

The occurrence of Prussian blue outside of the gastric tubules in the blood and lymph vessels and in the connective tissue spaces, Miss FitzGerald attributed as due, possibly, to a certain toxic action of one or other of the injected salts on the gland cells, causing the discharge of free acid from the parietal cell in an inward, as well as outward, direction. From her results she drew the following conclusion:—"The occurrence of Prussian blue reaction in the canaliculi of the parietal cells affords conclusive evidence of the presence of free acid within these structures." Although having no very definite evidence on the point, she was of the opinion that the hydrochloric acid was formed free within the cytoplasm of the parietal cell itself, from whence it diffused into the canaliculi. She thought such a view to be more tenable than one which supposed the hydrochloric acid to be formed free in the canaliculi only, and the parietal cell body itself to be alkaline.

Corroborative evidence that the parietal cells were the seat of the elaboration of the free hydrochloric acid of the gastric juice was also obtained by Miss FitzGerald. Greenwood (1885), whose work has been referred to earlier in this paper, found that the parietal cells could be differentiated from the chief cells after fresh gastric mucosa had been treated with a

solution of silver nitrate. Professor Macallum (1905) showed that the staining properties of silver nitrate solutions were due practically entirely to the formation of insoluble silver compounds in the tissue when it was penetrated by the nitrate reagent. These silver precipitates became subsequently transformed by the action of light into coloured reduction compounds, and it was these that imparted the characteristic colours to sections which had been treated with the nitrate of silver. The silver salts, which were thus precipitated in the tissue, consisted almost wholly of chlorides, phosphates and carbonates. He devised also a method for the localization of the chlorides micro-chemically. When a solution of silver nitrate containing 1.5 per cent. of free nitric acid was utilized, he showed that, with the exception of taurine and creatine, only the chlorides in the tissues would be precipitated. As taurine and creatine are not present in the majority of the tissues, or at least never in any appreciable amount, a specific reagent for the detection and localization of chlorides in any tissues was thus made available.

Miss FitzGerald placed in silver nitrate solutions and the special chloride reagent, respectively, portions of the fresh gastric mucosa. The vessels containing the solutions were then exposed to sunlight for some time. Portions of the tissue were then teased out and mounted on slides in glycerine. She found that the parietal cells were clearly defined in both instances. The amount of the reduced silver compounds observed in the chief cells and the interstitial tissue was relatively very much less than that in the parietal cells. The reduction manifested in the parietal cell was more marked when pure nitrate of silver solution was the reagent used. The abundance of chlorides, phosphates and carbonates in the parietal cells, thus shown, was put forward by Miss FitzGerald as evidence further indicative of the acid-forming function of these cells.

Harvey and Bensley (1912) repeated much of Miss FitzGerald's work. They also introduced many new methods of study. They did not think that Miss FitzGerald's results had settled the question, and held that all results obtained by the

Prussian blue method would have to be ruled out of consideration. They, themselves, obtained results similar to Miss FitzGerald's by this method, but they found, as well, Prussian blue in practically every tissue of the body. They claimed, therefore, that the Prussian blue, which was found in the gland tubules and the canaliculi of the parietal cells, had not necessarily been precipitated there, but might have been formed elsewhere, and then have been transported thither by means of the blood and lymph streams. How this transport of Prussian blue through membranes and cells could obtain they did not explain for even the "soluble" form of this compound does not penetrate these structures. Further, the solutions of sodium ferrocyanide and ferric ammonium citrate used by them were *of exceedingly greater concentration than those used similarly by Miss FitzGerald.* The latter in her experiments had injected only very small quantities of a "balanced" preparation, which she prepared by mixing equal parts of a 2.25 per cent. solution of ammonium ferric citrate and 1.5 per cent. solution of potassium ferrocyanide. Harvey and Bensley used, however, at times *twenty-five per cent. solutions of ammonium ferric citrate and ten per cent. solutions of sodium ferrocyanide, mixtures of which, without the addition of any acid, give copious precipitates of Prussian blue.* These they injected separately. They never found Prussian blue in the parietal cells without finding it also in the blood vessels, while in some cases they found it in the blood vessels without finding it in the parietal cells.

Deposits of Prussian blue were obtained on the surface of the mucosa of the proventriculus of the fowl, and also in depressions leading to the compound glands of the same. They did not observe any in the cells or the lumina of these glands, but found them in the adjacent blood vessels and lymph spaces, as well as elsewhere, especially in the endothelial cells of Kupffer in the liver. In the turtle Prussian blue deposits were produced in the epithelial cells of the foveolae and the neck cells of the gastric gland tubules to a certain extent and in the cells of the bodies of these glands to a slight extent. The reaction

was obtained in the connective tissue and blood and lymph vessels as in the cat, dog and fowl, and it occurred similarly in the skate.

Experiments designed to show the effect of injury of the gastric mucosa, of the presence of poisons, and of restricted blood supply on the Prussian blue reaction, gave more or less negative results. Parietal cells in the region of injury in the dog and cat were found to be loaded with Prussian blue. Restricting the blood supply of the mucosa caused, in some cases, ulcers to arise, and these were found always to be coated with Prussian blue.

The occurrence of Prussian blue in many tissues, such as the blood, lymph, liver, spleen, intestine, etc., proved, they claimed that the reaction probably took place without the help of an acid. They suggested that some such factors as the withdrawal from the blood stream of the ammonium ferric citrate more rapidly than the sodium ferrocyanide, owing to its easier diffusibility or the involvement of the ammonium salt in the metabolic processes of the tissues, or the death or injured vitality of some of the cells, might furnish an explanation of its widespread occurrence.

By the use of a number of aniline dyes, particularly of cyanamin bichloride, they endeavoured to determine the localization of the acid formation in the gastric mucosa. In the presence of an alkali this dye becomes red, while in neutral or acid solutions the colour is blue. When portions of gastric mucosa, removed from actively digesting stomachs, were teased out in saline solutions of the dye the canaliculi in the parietal cells stained red, the lumina of the glands were bluish red and the canals connecting the canaliculi and the lumina were intermediate in colour, while the secretion as it obtained in the foveolae and even the cylindrical cells lining them took on a blue colour, all in contrast with the results observed in the gastric mucosa of the resting stomach which did not give these reactions.

They obtained somewhat similar results with Nile blue and neutral red. The latter dye is red in neutral solution and

crimson in acid solution, but when either is rendered alkaline a yellow base is precipitated. When portions of the freshly removed gastric mucosa, actively secreting, were teased out in a solution of one part of this dye in ten thousand of saline solution the canaliculi of the parietal cells were yellow, the glandular lumina red and the canals connecting were of an intermediate colour.

These results led them to conclude that the hydrochloric acid was not free as such in the gastric tubules, but was liberated in the foveolae, and in the epithelial cells of the surface of the gastric mucosa. The parietal cells and the contents of their canaliculi they held to be alkaline. The parietal cells, they were inclined to hold, elaborated a compound, possibly a chloride of an organic base, of protein character, which gives rise to free hydrochloric acid when it diffuses into the foveolae and upon the surface of the mucosa.

Hammett (1915) has criticized the evidence which had been put forward by both Miss FitzGerald and Bensley and Harvey in regard to the source of the hydrochloric acid in the stomach. He held that the reasons which led Bensley and Harvey to reject the results obtained by the use of the Prussian blue experiment were not justified.

Their criticisms concerned, amongst other results, the inconstancy of the reaction itself in the stomach on all occasions, and when it was obtained, its restriction to limited areas within which only a few of the parietal cells reacted. Hammett holds that regional activity, decreased blood supply and the toxic effects of the injected salts could account for some of these results, and that the non-reacting cells amongst the reacting ones could be considered as in the resting state, or to have already discharged their acid and by the toxic action of the injected salts to have been prevented from further activity.

Their most weighty objection was that the reaction occurred in other organs than the stomach. He attributed this result to the presence of an acid, such as lactic, in the tissues, especially in heart muscle when it is dead or dying, which

would explain the occurrence of Prussian blue in this tissue, as observed by Harvey and Bensley, when none was found inside the blood vessels of this organ.

Hammett maintained, contrary to Harvey and Bensley's results and conclusions on this point, that when solutions of sodium ferrocyanide and ferric ammonium citrate are mixed with blood or blood serum *in vitro* Prussian blue does not form, but he does not indicate the concentration of the solutions he used. It is, however, probable that he used concentrations of these salts not greater than those used by Miss FitzGerald.

Hammett repeated Harvey and Bensley's experiments with cyanamin. This dye, as already explained, gives distinctive reactions for acid, alkaline and neutral solutions. With its use he got the same results as they did, but he points out that the parietal cells, if they secrete acid, should be, as was found to be the case, alkaline in reaction, and further, that in pieces of the fresh mucosa removed and treated with this dye secretion ceases and the distribution of the dye in the structures depends on the relative velocity of diffusion of the dye and of the acid which is greater than that of the dye. As the rate of diffusion of the acid is greater it is only where the dye and acid freely mingle, as for example, on the surface of the mucosa and in the foveolae, that the acid reaction is definitely indicated, whereas in the lumina of the gland tubules and in the canaliculi connecting the lumina and the parietal cells there is no interaction of acid and dye because the dye does not diffuse into them.

The results, therefore, Hammett maintains, do not contradict Miss FitzGerald's observations and conclusions which he regards as confirmed by his own and Harvey and Bensley's results.

## II. METHODS OF STUDY

A great deal of the work in microchemistry in the past has been done under considerable difficulty. The results had nearly always to be interpreted as involving the possibility that a slight shifting or an extending of the area, to which the substance being localized was confined, had taken place. This shifting could be brought about by diffusion and osmosis. These might possibly exert a certain action in the time between the removal of the tissues from the body and the penetration of the specific reagent used to the innermost parts of the same. The nearer, therefore, one can approach conditions under which the precipitating reagent is brought into immediate and intimate contact with all parts of a tissue in which the various constituents are still in their normal relations, the greater accuracy in the localizing of any constituents will thereby be attained.

The freezing microtome is indispensable for this purpose. With it, from tissues excised immediately after the death of the animal, sections can be prepared without any delay in which there is practically no alteration in the distribution of the salts as they occur in the normal living structures. The sections so prepared must, however, be transferred while frozen to the reagent to be used on them, for if they are allowed to thaw changes will obtain which will tend to give confusing results. To prevent this thawing of the sections the sectioning knife and the air about the microtome must be kept at a temperature below 0° C. This was effected by enclosing the microtome in a wooden box lined on the bottom and sides with asbestos and covered by a thick sheet of plate glass, into which box led two tubes, each terminating with a spray nozzle from a drum cylinder of liquid carbon dioxide, one to use in freezing the piece of tissue on the stage of the micro-

tome, the other provided with a movable joint connection, to spray the knife as occasion required, and also to chill the air. To permit the microtome thus enclosed to be worked, an opening in each of two opposite side walls permitted the introduction of the hands of the operator. With the dish containing the reagent to be used, and with a camel's hair brush chilled to the temperature of the air in the box it was possible to transfer the sections made, while still frozen to the contents of the dish. This reduced to an absolute minimum the risk of the constituents, inorganic or organic, diffusing from the structures in which they are held in the normal tissue.

For the localization of inorganic constituents in the tissues thus sectioned special methods were employed. These and others which were also employed in this research will now be described.

1. *The methods for the detection of chlorides and phosphates.* The methods for studying the distribution of the chlorides and the phosphates and carbonates will be considered together. The special reagent for the former consisted of a decinormal solution of silver nitrate which contained 1.5 per cent. free nitric acid, that for the latter consisted simply of a decinormal solution of silver nitrate (Macallum, 1905). These two reagents, when used in conjunction, enable one to study the distribution of, and the relative relations existing between, the chlorides on the one hand, and the phosphates and carbonates on the other, in any tissue.

The distribution of the chlorides can be definitely determined, while that of the phosphates and carbonates can be quite accurately decided by the method of difference. Whether the excess reduction, which is observed in the latter case, is due entirely to phosphates or in part to carbonates cannot be determined, for there is no microchemical reaction for the latter alone. These two inorganic constituents of the tissue are, however, probably nearly always associated one with the other, and the results obtained with silver nitrate alone, when allowance is made for the presence of chlorides, should, there-

fore, be held to indicate the presence of carbonates as well as phosphates.

The tissue (which consisted of typical portions of the mucosa of various vertebrate types) was always removed from the animal as quickly after it was killed as circumstances would permit. The larger animals were always anaesthetized, the smaller ones pithed. In every case the stomach was immediately exposed and the portion of the mucosa removed, frozen and sectioned after the method previously detailed. When either the decinormal solution of silver nitrate or the "chloride reagent" was used, the sections were allowed to remain in a dark cupboard for a few hours in the respective reagent. The sections were then removed from the silver solutions by means of large carefully cleaned glass rods and transferred to dishes containing distilled water. From this they were taken up on slides which were then drained, a drop of 50 per cent. glycerine was applied to each section and a cover slip placed over it. The mounted preparations were then placed in bright sunlight for a few hours. This completed the process. The preparations were made permanent by luting the edges of the cover slips with Canada balsam.

2. *The method for the detection of phosphates alone.* The nitric-molybdate reagent introduced by Professor Macallum (1898) for the localization of phosphorus, chiefly that organically combined in animal and vegetable tissues, can also be utilized for determining the distribution of phosphates, with more or less success, if applied in a special manner. This reagent is made by dissolving one part of pure molybdic acid in four parts of ammonia and then adding this solution slowly to fifteen parts of nitric acid of specific gravity 1.2. The prolonged action of the nitric acid will convert part of the organically combined phosphorus into the ortho-phosphate, and this in the presence of the reagent is precipitated as ammonium phospho-molybdate, which is yellow in colour, but becomes dark green when it is "reduced" by treatment with a 1 per cent. solution of phenylhydrazine hydrochloride. If, however, the sections are treated with the nitric-molybdate reagent for

a period of 5 to 10 minutes only, and at a temperature of about 40° C., only the inorganic phosphate present will be precipitated. Further treatment consists then in the reduction of this precipitate by a one per cent. solution of phenylhydrazine hydrochloride. This requires but a few minutes. The sections are then washed and mounted on slides in 50 per cent. glycerine. The distribution in the section of the dark green "reduced" compound indicates the distribution therein of phosphate salts.

3. *The method for the detection of potassium.* The method for studying the distribution of potassium embodies the use of the cobalt hexanitrite reagent (Macallum, 1905). This reagent is made by dissolving 20 grams of cobalt nitrite and 35 grams of sodium nitrite in 75 c.c. of dilute acetic acid (10 c.c. of glacial acetic diluted with distilled water to 75 c.c.). The solution of filtered and is then made up to 100 c.c. with distilled water. Sections are treated with this reagent for but a few minutes. The potassium is precipitated as the triple salt, cobalt sodium potassium nitrite, $Co(NO_2)_3 3(KNa)NO_2 + nH_2O$, $n$ being either 1, 2, or 3 (Gilbert, 1898). This salt is insoluble in ice-cold water which is, therefore, used in washing the sections until they are quite free even of traces of the reagent. They are then mounted on slides in a mixture consisting of equal parts of ammonium sulphide and 50 per cent. glycerine. The distribution of potassium corresponds with that of the black sulphide of cobalt which results from the action of the ammonium sulphide on the triple salt wherever it obtains in the preparations.

4. *The "Prussian blue" method.* Small quantities of a "balanced" mixture of sodium ferrocyanide and ammonium ferric citrate, made by adding 10 parts by volume of a 3 per cent. solution of the former to 7 parts of a 4 per cent. solution of the latter, were allowed to flow slowly into a vein from a burette. The solutions were always freshly prepared, and when injected were tepid. After the injection and an interval which varied in different cases, the animal was killed. Small portions of the various organs were removed, fixed in

absolute alcohol for a period of at least 24 hours, then passed through chloroform in the usual way to hard paraffin and sections of them of 5-10 $\mu$ in thickness were then made, which were slightly stained with eosin and mounted in Canada balsam.

Soluble Prussian blue was also injected into some animals intravenously. A one per cent. solution of this substance was employed. The method of procedure following the injection was exactly the same as that just detailed for the "Prussian blue" experiments.

5. *Polychrome B as an acid indicator.* This dye which gives a reddish-brown colour when added to neutral or alkaline aqueous solutions, is precipitated as a violet-coloured compound when a solution of it is rendered acid in the slightest degree. Acid salts such as sodium di-hydrogen phosphate, when added to a neutral solution of this dye, will also effect its precipitation. It was injected intravenously into some animals. A 0.7 per cent. solution was used and only small quantities were given. In large amounts it proved to be very toxic. The precipitate is somewhat soluble in water, alcohol, and to a certain extent, also, in glycerine, so that no permanent preparations of tissue, from animals treated thus with this solution, can be made. Sections made by the freezing method, of portions of the freshly removed gastric mucosa of the animals were at once mounted direct from the sectioning knife, in glycerine, and immediately examined under the microscope.

6. *Histological fixation and staining methods.* In these, saturated solutions of mercuric chloride in water or, as in Bensley's modification of Foa's blood-fixing fluid, in alcohol with equal volume of 3 per cent. aqueous solution of potassium bichromate were used. Preparations fixed with the former were hardened in it for 24 hours, those made with the latter were kept in it for one to two hours. The dehydration and the sectioning, staining and mounting were carried out in the usual way. Absolute alcohol alone was also used as a fixative.

## III. RESULTS

The results obtained with the different classes of the Vertebrata studied will now be described, in detail.

### *Mammals*

The chief Mammalian types studied were the dog, cat and rabbit. Some difficulty was experienced in keeping animals in a normal condition, as well as in getting them into the particular state desired for any definite experiment. Pavlov's "psychic factor" in digestion was noticeable in several cats. The fear which they showed when they were in the same room with dogs seemed to inhibit quite effectively their gastric secretion. Rabbits are often very unsatisfactory to use as it is only rarely that their stomachs are found empty.

### *The Resting Mammalian Stomach*

The application of the method for chlorides demonstrates only an almost negligible quantity of the same in the gastric tubules of the fundus region of the resting stomach. *In both the chief and parietal cells chlorides are present in traces only. The interstitial tissue, on the other hand, is very rich in these, and there is also a heavy condensation of such salts about the gland tubules.* The lumina of the tubules may contain a slight amount.

Sections, treated after the method for phosphates and carbonates, stand in marked contrast with those prepared to show the localization of the chloride. A distribution of these salts, similar to that of the chlorides, manifests itself in the connective tissue, cells and fibres, and about the outer border of the tubules, as well as to a slight extent in the lumina. *The parietal cells, however, stand out in a remarkable manner. They are seen to contain an exceedingly voluminous deposit of reduced silver compounds, clearly indicating a very great content of phosphates and carbonates on the part of the parietal cells. Practically every parietal cell seems to be picked out in this typical manner, while the chief cells exhibit only slightly more reduction than they had in those sections which*

*had ben treated for chlorides alone.* The inorganic salt constituents of the parietal cells are confined entirely to the cytoplasm of the same, as in no instance has any such salt been observed to be present in their nuclei.

*The Active Mammalian Stomach*

In the fundus region of the actively secreting mucosa there is a condensation of the chlorides on the external faces of the tubules and in the interstitial tissue, similar to that indicated above, as well as an appreciable deposit in the lumina of the tubules. The chief cells of these are quite free from chlorides as they are in the resting condition. *On the other hand the parietal cells, in areas more or less discontinuous, are extraordinarily rich in chlorides and the canals connecting these cells with the lumen in each tubule are clearly and sharply outlined by the chloride reaction developed in them.* Outside these areas, however, some groups of parietal cells, more or less limited in number, may give no more marked a reaction for chlorides than do those in the glands in the inactive condition. It is very probable that the tubules in which this occurred had not begun to secrete.

The use of the method to detect the occurrence of phosphates and carbonates reveals the same concentration of these salts in the parietal cells that obtains in them during their resting stage.

The occurrence of the reduced silver compounds in the parietal cells is generally in the form of very fine granules closely approximated to one another and distributed throughout the cytoplasm of the cells. It is, however, to be noted that, in the immediate vicinity of the nuclear membrane and to a much less extent within the cytoplasm immediately adjacent to the inner or luminal border of such cells, there is a greater condensation of these granules than elsewhere in the cytoplasm. This peri-nuclear condensation is, in a way, very remarkable, and was observed to be present whether the salts present were those of chlorides alone, or of these and the phosphates and carbonates. So marked is this condensation, at

times, that the granules constituting it have the appearance of forming a solid envelope of reduced silver compounds enclosing the nucleus.

The application of the cobalt hexanitrite method for potassium demonstrates that this element is present only in traces in the gastric mucosa, while the parietal cells appear to be quite free from it. *This would indicate that the inorganic salts which are present in the parietal cells are not those of potassium, but consist probably very largely of compounds of sodium.*

The use of the nitric-molybdate reagent on the frozen sections showed that phosphates were abundant in the parietal cells.

A rabbit, which had been kept from food for two days, was allowed to partake of a hearty meal of oats. One-half hour later 20 c.c. of a 0.7 per cent. solution of Polychrome B was injected in the auricular vein. No toxic symptoms were manifested by the animal. One and a half hours after the injection had been given the animal was killed. Sections prepared after the method earlier set forth showed on examination a diffuse blue colour in the parietal cells. This was particularly in evidence in the portion of the cytoplasm adjacent to the inner or luminal border of each cell. The parietal cells of the upper third of the bodies of the gastric tubules of the fundus region were in this manner specially demonstrated. It is probable that this reaction is due to the presence in these cells of the acid phosphate of sodium ($NaH_2 PO_4$) which, like a free acid, give a blue violet precipitate with the reddish brown solution of this dye.

Neutral red was also used. Frozen sections of the mucosa of active stomachs were dropped into dilute solutions (1:10000) of this dye. They were mounted after a few minutes on slides in 50 per cent. glycerine. Sections were also placed in dry slides and a drop of neutral red applied. Five minutes were allowed for staining to take place, then a drop of glycerine was added and a cover slip placed over the section. Both of these methods yielded similar results. The re-

sults were, however, too indefinite to draw any specific conclusion. The parietal cells were not observed to take on the alkaline shade of the dye, nor did the canaliculi. A diffuse red staining throughout the section was rather the condition observed.

*Birds*

As the proventriculus of birds contains the glands which correspond with those which are to be found in the fundus region of the gastric mucosa of the Mammal, preparations from this organ in the hen were used in obtaining the results which are now to be detailed. The hen was chosen to represent this class of Vertebrates because in it the organ is typical of the Avian proventriculus, and also because the animal could be made to undergo the conditions required in the experiments without difficulty.

*The Resting Proventriculus of the Hen*

*The chlorides are in great abundance condensed in the intertubular tissue of the compound glands and around the tubules themselves.* The lumina of the tubules are not much in evidence in preparations made to show the distribution of the chlorides. The gland cells are for the most part quite free from chlorides, but a few cells manifest a slight chloride content which, however, is quite uniformly distributed throughout their cytoplasm. Occasionally a small restricted area in sections of the organ can be found in the cells of which there is a slight condensation of chlorides. The latter are chiefly localized in that portion of the cytoplasm adjacent to the luminal border and the neighbouring margins of the cell. As will be indicated later, these restricted areas constitute small groups of actively secreting gland cells.

The results of the use of the method for demonstrating the phosphates and carbonates show that these are abundant in the connective tissue and also about the tubules in the form of lymph condensations, *but the portion of the cytoplasm of each gland cell adjacent to the lumen, equivalent approximately to one-third of its volume, manifests a remarkable*

*saturation with these salts.* So heavy is the deposit in this region that under a comparatively low magnification the lumina appear to be bounded by a solid black "reduced" silver deposit of irregular outline. External to these deposits and distal to the lumen, the cytoplasm of each cell is free of any deposit, but this clear zone is, in its turn, bounded externally by the reddish brown deposit due to a "reduced" silver deposit in the lymph condensation of the various salts about the tubules. *As in the case of the parietal cells of the resting Mammalian stomach this condition is remarkable on account of its uniformity of occurrence in all the gland tubules of the resting proventriculus.*

*The Active Proventriculus*

The use of the method for chlorides demonstrates the usual condensation around and between the tubules, but the cells, unlike those of the resting gland, are well defined. *They have a marked condensation of chlorides within them which is almost entirely confined to a narrow zone in the cytoplasm of each cell adjacent to its luminal border, and to the contiguous margins extending outwards towards the basal borders.*

*Treatment with the method for phosphates and carbonates also indicates a similar distribution of these salts.* Under low power magnification they appear to be condensed along the luminal borders of the gland cells and from this zone of condensation deposits appear to extend between the gland cells towards their basal borders. Under the oil-immersion objective, however, the heavy black deposit is seen to be confined entirely to the cells themselves, localized within each cell in a sharp and clear-cut manner, as already indicated.

*In the active proventriculus, therefore, the chlorides, phosphates and carbonates are found to have a like distribution in the cytoplasm of the gland cells.* The phosphates and carbonates are, of course, considerably in excess of the chlorides and occur in the position just described quite uniformly in the cells throughout the glands. The distribution of chlorides in

these typical positions, while quite extensive, is not, however, as uniform as that of the phosphates and carbonates.

The Prussian blue experiment, the complete method of procedure for which has been previously outlined, was carried out on hens quite successfully. The quantity of "balanced" mixture injected varied in amount between ten and twenty cubic centimetres. The brachial vein was found to be the most serviceable for injections. A slight toxic effect was usually manifested, but this did not appreciably affect the animals as they would always continue to peck up grain offered to them after the injection had been given. The injection was, in all cases, made three hours after the fowl had fed. One hour after the injection had been given the proventriculus was removed, opened, carefully examined and small portions removed and fixed in absolute alcohol. The contents of the muscular stomach, as well as the surface of the mucosa of the proventriculus, were in all cases found to be acid in reaction. A deposit of Prussian blue was always observed on the mucosa of the wall of the proventriculus. In some instances this deposit was very heavy and appeared to cover the entire surface of the mucosa throughout the length of this organ. In other cases the Prussian blue precipitate was confined chiefly to the mouths of the papillae while the mucosa between these showed a light deposit of varying intensity. Microscopically, also, Prussian blue could be occasionally seen in the large ducts of the compound glands when free hand across sections were made of the proventriculus. Microscopic examination of alcohol-hardened tissue, sections by the paraffin method and slightly counter-stained with eosin, showed that Prussian blue was present in the mucosa immediately adjacent to the heavy surface deposit. It occurred in slight traces in the interstitial tissue of the tubules of the compound glands. It was also present in the lumina of these tubules as well as in the large collecting tubules and the necks and mouths of excretory duct opening immediately by the papillae. The gland cells of the tubules never manifested a deposit of Prussian blue within them. As just stated, however, a deposit was to be

observed at times in the lumina of the tubules. In such cases it was present either as a solid plug, practically filling the lumen, or *else it occurred in the form of a fine granular precipitate which followed the contour of the luminal wall.* Whether in this latter case the deposit was actually within the gland cells or condensed only on the outside was difficult to determine.

The Prussian blue was not confined to the proventriculus, however. Very slight traces of it were found in the connective tissue of both the pancreas and the intestinal tract. Heart, lung and spleen were free from it as a rule, while in the liver it was observed in the endothelial cells of Kupffer.

In order to determine how Prussian blue, which might be circulating in the blood stream, would be finally disposed of, 14 c.c. of soluble Prussian blue were allowed to gravitate into the brachial vein of a fowl. No untoward symptoms were manifested by the animal. After 40 minutes had elapsed the animal was anaesthetized and portions of the various tissues were immediately placed in absolute alcohol. A small quantity of a light blue fluid was present in the cloaca, otherwise no blue was seen macroscopically. Microscopic preparations of the various tissues prepared according to the method earlier indicated, demonstrated a certain amount of Prussian blue to be present in the connective tissue, the blood-vessels and the lymph channels. The only place where it was observed to be at all abundant was in the endothelial cells of Kupffer of the liver. The proventriculus was no exception to the general statement just made. Prussian blue was observed in its interstitial tissue as in other organs. It was very worthy of note, however, that there was no deposit of Prussian blue on the surface of the mucosa nor in any of the gland cells, nor was such a deposit observed in the lumina of any of the tubules.

### *Histological Findings*

The proventriculus of a fasting fowl was fixed in Bensley's fluid and preparations finally made according to Method 6. The luminal portion of each gland cell in these preparations

was found to be markedly stained with eosin, while the outer or basal portion of each cell was not so stained, and they presented a fibrillar appearance and a diffuse haematoxylin stain. In the active proventriculus there was no such differentiation with eosin in the cytoplasm which was stained uniformly throughout. The nucleus, which, however, did not stain with eosin, was observed to be richer in chromatin material than in the resting cells. The cells themselves were perhaps slightly smaller in the active than in the resting gland.

When other fixatives were employed such differentiations as these could not be distinguished so readily.

*The Turtle*

The condition manifested in the tubules of the fundus glands of the stomach of the turtle is very similar to that in the fowl. *In the gland cells in the resting condition the cytoplasm of the half of each cell adjacent to the lumen is very rich in phosphates and carbonates,* not a trace of which is to be found in the remainder of the cytoplasm. The boundaries of the cells can be distinguished only with difficulty. The gland tubules are for the most part quite free from chlorides, but, as in the hen, a few scattered cells contain them. These salts are uniformly distributed in the cytoplasm of such cells.

The results of the use of the method for demonstrating the phosphates and carbonates in like preparations from the stomach in the active stage *show that these are present in the cells of the gland tubules, but more sharply defined in the portion of the cytoplasm of each cell immediately adjacent to the lumen than is the case in the cells of the resting glands. The reaction of chlorides shows a similar distribution.* The condensation of all these salts in the lymph about the tubules and in the interstitial tissue can be clearly demonstrated in all cases.

*The Frog*

*The distribution of the chlorides, phosphates and carbonates in the gland tubules of the fundus region of the frog's stomach* (*the oxyntic glands of Langley*, 1881), *corresponds,*

*also, very closely with that observed in the hen. In the resting glands the tubules are almost devoid of chlorides.* Here and there a mere trace is manifested in the lumina of the tubules. The interstitial tissue is, in every case, abundantly supplied with chlorides, and there is the usual condensation of chlorides in the lymph about the tubules. Phosphates and carbonates are particularly abundant in the portion of the cytoplasm of each cell adjacent to the lumen. As in the fowl, the remainder of the cytoplasm is free from these salts. The remarkable absence of chlorides from these cells had been previously noted by Professor Macallum (1908).

*The active stomach of the frog, while manifesting a different condition from that of the resting organ does not present such a definite localization of the chlorides, phosphates and carbonates as is to be observed in the hen. The chlorides are present in the lumina and in the cytoplasm immediately adjacent. Apart from this luminal condensation the gland cells are free from chlorides as in the resting organ. The distribution of phosphates and carbonates is, however, sharply defined, and is confined to the cytoplasm in the luminal half or third of each cell.* The portions of each cell on their contiguous borders are outlined to a greater or less extent by the condensations of these salts in them.

## IV. GENERAL DISCUSSION

The general significance of the results of the investigation given in the preceding pages may be now discussed.

There never was any doubt about the participation of the chlorides, especially that of sodium, in the reaction in the gastric mucosa by which the hydrochloric acid of the gastric juice is formed. Sodium chloride is the most abundant mineral compound in the blood and lymph, and it must accordingly be the source of the chlorine of the acid. The problems which had to be solved were the nature of the reaction which produced the acid from the chloride and the kind of cellular elements in the mucosa in which the reaction occurs.

It is pertinent here to refer to some views as to the character of the reaction to which the findings of this investigation give special interest.

Maly (1874) obtained free hydrochloric acid by acting on lactic acid with sodium chloride. He was of the opinion that it was in some such manner as this that the hydrochloric acid of the gastric juice was formed. Later, however, he changed his views on this point (1878). This, he finally held, was not the normal method for the formation of the hydrochloric acid. It might be the method to a certain extent in dyspeptics, but he held that in the normal stomach the hydrochloric acid is liberated through the mass action between sodium di-hydrogen phosphate and sodium chloride in the cells of the gastric mucosa. The blood, though alkaline, contained acid salts and so such a reaction would be possible. The free acid would have to be swept away as fast as it was liberated, while the supply of acid phosphate would be kept up by the action of free carbon dioxide on the basic salt formed along with the hydrochloric acid. Maly's theory may, therefore, be expressed by the two equations:

1. $NaH_2PO_4+NaCl=Na_2HPO_4+HCl$.
2. $Na_2HPO_4+CO_2+H_2O=NaH_2PO_4+NaHCO_3$.

He noted also, that the urine of dogs during gastric activity was strongly alkaline. He thought that the degree of alkalinity of the blood was normally kept constant, and so when, in any tissue, free acid was being elaborated from the blood, the acid secretion of the kidneys would necessarily lag behind.

Von Noorden (1886) estimated the amount of the carbon dioxide in the arterial blood before and after feeding. He could not detect any regular increase in the alkalinity of the blood by this method. He concluded that the secretion of acid in the stomach had nothing to do with the alkaline tide observed in the secretions from the kidney during the period of gastric digestion.

Bunge (1889) held that the hydrochloric acid was formed by the interaction of the parietal cells according to the method indicated by Maly. Carbon dioxide, he said, would be avail-

able free, by the action of a ferment, or by the oxidation of organic combinations.

The results obtained from the study of the distribution of the chlorides, phosphates and carbonates in the gastric tubules of the various Vertebrate types of the stomach during the different stages of their activity justifies the adoption of the Maly theory as to the mode of origin of the hydrochloric acid. In the Mammalian stomach, where two types of gland cells exist in the bodies of the gland tubules of the fundus region, the distribution of the various salts shows that the acid-elaborating function belongs to the parietal cells. The absence of chlorides in these during their resting stage and their presence in them while in the active stage is of unmistakable significance in this respect. These cells, during rest and activity, appear to possess the ability to store up and retain phosphates and, very possibly, carbonates. Chlorides, however, are not taken up by the parietal cells until they are to "go into action." The latter are then admitted and continue to be admitted as long as the gland cells are secreting. There is probably at the same time a high carbon dioxide concentration in the cell or its immediate vicinity to ensure an adequate supply of the acid phosphate. It is not probable that the hydrochloric acid occurs in any quantity in the parietal cells themselves, but diffuses as quickly as it is formed into the intracellular canaliculi from whence it passes by the diverticula of the lumen into the latter.

The findings from the method embodying the use of Polychrome B indicate that the parietal cells contain, during their active state, either free acid or acid salts. The absence of the faintest trace of a deposit of Prussian blue in parietal cells when a "balanced" mixture of solutions of sodium ferrocyanide and ammonium ferric citrate is injected into the circulation makes it certain that free acid does not occur in their cytoplasm. It is, therefore, very probable that the reaction with Polychrome B was due to sodium di-hydrogen phosphate, the presence of which, as postulated in Maly's theory of the origin of the hydrochloric acid of the gastric juice.

In the light of all the evidence which has been considered, Miss FitzGerald's findings are confirmed, and her interpretation of them must be accepted. Attempts to minimize their significance, it seems, have not been fully justified.

In the fowl we saw the same tendency on the part of the gland cells to store up phosphates and carbonates as is manifested in the Mammalian parietal cells. The typical condensations of chlorides, phosphates and carbonates in the gland cells of the gastric tubules of the active proventriculus, which have been described, and the absence, for the most part, of chlorides from these cells during the resting state would, taken in conjunction with the results of the injection of the "balanced" mixture of solutions of sodium ferrocyanide and ammonium ferric citrate, indicate that the hydrochloric acid of the gastric juice of the hen is formed by the glandular cells of the tubules of the compound glands, and that it is liberated either immediately within the cell border or at the cell margin and diffuses into the gland lumen. The cytoplasm of these cells possesses the power, when stimulated to activity, to concentrate large quantities of chlorides, phosphates and carbonates in the zones which are adjacent to the luminal border and the contiguous sides. Such facts would indicate that the hydrochloric acid is formed as a result of the mass action between these salts.

In considering the significance of the results of the injection, in the hen, of the "balanced" mixture of solutions of potassium ferrocyanide and ammonium ferric citrate it is well to remember the relatively great amount of hydrochloric acid present on the surface of the mucosa in the active proventriculus. A certain amount of this may, under special conditions, undergo reabsorption by the mucosa. This would account for the occurrence of Prussian blue external to the cells secreting the acid. The same observation may be applied to explain irregularities observed in the occurrence of Prussian blue in other orders of the Vertebrata. Further, the presence of lactic acid, *e.g.*, in striated muscle and the toxic action of these salts in certain concentrations, which may alter the direction

of the current of the secreted acid, may very possibly account for other peculiarities in the distribution of the Prussian blue observed. The occurrence, also, of the reaction on the endothelial cells of Kupffer is intelligible. These cells, as Professor Macallum (1895) found, absorb in an unusual degree the salts of iron in the blood stream passing them and, if they absorb potassium ferrocyanide as readily, Prussian blue must by mass action form in them as it does when concentrated solutions of potassium ferrocyanide and ammonium ferric citrate are mixed in a test-tube.

The production of Prussian blue in tissues elsewhere than in those of the gastric mucosa without the participation of an acid in its formation, therefore, does not destroy the value of the "balanced" mixture of potassium ferrocyanide and ammonium ferric citrate, when appropriately used to determine in what cells of the peptic tubules hydrochloric acid is formed.

The absence of chlorides in the parietal cells at points or in restricted zones in the actively secreting stomach, while surcharged as such cells are with phosphates and carbonates, indicates only that not all the gastric glands are in full secreting activity at any one time. This is quite in accord with the observations of Brown (1912) on the excretion of potassium salts by the kidney, who observed that while groups of the renal tubules were engaged in this excretion other groups here and there in their immediate neighbourhood appeared wholly inactive in this respect. The assumption, therefore, that all the cells of a glandular organ when it is secreting are in action at the same time, is not justified by the facts so far ascertained.

A brief survey of the results obtained in the turtle and the frog makes it clear that these are in line with those obtained from the mammal and the fowl. The fundus gland cells of the turtle and the cells of the oxyntic glands of the frog contain, at all times, great quantities of phosphates and, very possibly also, carbonates. Chlorides were comparatively rare during the resting periods with the exception of a few odd cells in which they were manifested to a certain extent. Dur-

ing activity the condensations of the two (or three) classes of salts seem to occur in the gastric glands cells with characteristic uniformity throughout all the lower Vertebrates.

Why the acid-secreting cells of the Vertebrate stomach are free from chlorides during rest and charged with them during activity is a question to which no answer can as yet be given. Doubtless the permeability of the cells to chlorides during activity is much greater than during rest, but how the change in permeability is effected is unknown. Equally difficult to explain are the facts that the acid set free in the cells normally moves in one direction, and that every trace of the acid is swept out of the cells immediately it is formed. It is evident that physical forces, of the nature of which we at present have no conception, play a very important part in these processes.

## V. SUMMARY OF RESULTS AND CONCLUSIONS THEREFROM

The results of the application of the microchemical methods of investigation described in the foregoing pages may be summarized as follows:

1. Phosphates, and very probably carbonates, are very abundant in the cytoplasm of the parietal cells of the gastric tubules of the fundus region of the resting Mammalian stomach while chlorides are absent from these cells, or occur in them in traces only.

2. Chlorides, phosphates and, very probably, carbonates, are very abundant in the cytoplasm of the parietal cells of the active Mammalian stomach.

3. The chief cells of the glands of the fundus of the Mammalian stomach are practically free from these salts at all times, that is, during rest and activity.

4. The phosphates and carbonates and also the chlorides, when the latter are present in the parietal cells, are either distributed uniformly throughout their cytoplasm or, less commonly, condensed about their nuclei.

5. The interstitial tissue of the mucosa of the fundus of the Mammalian stomach is rich in these salts at all times and a condensation of these salts occurs in the lymph on the immediate surface of each peptic tubule. Upon this supply the parietal cells may draw as their activities determine.

6. The use of the dye Polychrome B demonstrates that the cytoplasm of the parietal cells in certain stages of activity is acid in reaction, probably owing to the presence of sodium dihydrogen phosphate.

7. Phosphates and, very probably also, carbonates are present in great concentrations in the cells of the gastric tubules of the resting proventriculus of the fowl and of the fundus region in the stomach of the turtle and frog. They are confined to and uniformly distributed in the half of each cell adjacent to the lumen, while chlorides which are present to a slight extent or in traces only are uniformly diffused throughout the cytoplasm of these cells.

8. Chlorides, phosphates and very probably also, carbonate are present in great abundance in the cells of the glands of the active proventriculus of the fowl and of the glands of the fundus portion of the active stomach of the turtle and frog, but their distribution in each cell is confined to the cytoplasm adjacent to the lumen.

9. The results of the injection, into the circulation of the fowl, of a "balanced" mixture of sodium ferrocyanide and ammonium ferric citrate indicate that hydrochloric acid is liberated as such on the luminal border of the cells in the gastric glands of the proventriculus of the fowl.

10. The foregoing facts give very strong support to Maly's theory of the formation of hydrochloric acid in the gastric mucosa. This theory postulates the interaction in the active acid-secreting cells of the sodium chloride and sodium dihydrogen phosphate, the products being hydrochloric acid and disodium hydrogen phosphate which latter is converted, as soon as it is formed, into the acid phosphate by the action of carbonic acid. The mono-sodium carbonate or the disodium carbonate which results must quickly pass from the cells into

the lymph and blood as its presence in the cells would at once terminate the reaction. The cells thus direct the acid they form in one direction, and the alkaline salts resulting from the reaction in another. How this is done is unknown, if surface tension forces are not involved. Equally unexplainable are the absence of chlorides during rest, their concentration during activity in these cells, and the remarkable condensation at all times, in them, of phosphates. Physical forces are, doubtless, involved which have not hitherto been found strikingly exemplified in non-living matter, perhaps because the conditions necessary to their manifestation do not occur therein.

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I wish in conclusion to express my thanks to Professor Macallum for the facilities provided in the Biochemical Laboratory of the University of Toronto for this research, and also for his kind supervision and helpful suggestions throughout the course of the same.

## EXPLANATION OF PLATES

### PHOTOGRAPHS

1. Transverse section of the mucosa of the fundus of the actively secreting stomach of dog. Chlorides, phosphates and carbonates shown in the parietal cells. $\frac{N}{10}$ $AgNO_3$—low magnification.

2. A portion of same more highly magnified.

3. Transverse section of the gastric tubules in the fundus of the inactive stomach of cat. Chlorides shown condensed in the interstitial tissue. $\frac{N}{10}$ $AgNO_3$+1.5 per cent. $HNO_3$.

4. Transverse section of the tubules in the active proventriculus of hen. Chlorides, phosphates and carbonates localized. $\frac{N}{10}$ $AgNO_3$.

### DRAWINGS

1. Transverse section of a gastric tubule in the fundus region of the active stomach of dog. Chlorides localized in the parietal cells. $\frac{N}{10}$ $AgNO_3$+1.5 per cent. $HNO_3$. x 1120

2. Transverse section of a tubule of one of the multilobular glands of the active proventriculus of hen. Chlorides, phosphates and carbonates localized. $\frac{N}{10}$ $AgNO_3$. x 1120

3. Transverse section of a tubule of one of the multilobular glands of the active proventriculus of hen. Chlorides alone localized. $\frac{N}{10}$ $AgNO_3$+1.5 per cent. $HNO_3$. x 1120

4. Transverse section of a tubule of the inactive proventriculus of

hen. Chlorides, phosphates and carbonates localized. $\frac{N}{10}$ AgNO x 1120

5. Transverse section of a gastric tubule of the inactive proventriculus of hen. Chlorides alone are shown. $\frac{N}{10}$ $AgNO_3$ + 15 per cent. $HNO_3$ x 1120

6. Transverse section of a gastric tubule of the fundus region of the inactive stomach of turtle. Chlorides, phosphates and carbonates localized. $\frac{N}{10}$ AgNO x 1120

7. Transverse section of a gastric tubule of the fundus region of the inactive stomach of frog. Chlorides, phosphates and carbonates localized. $\frac{N}{10}$ $AgNO_3$ x 1120

8. Transverse section of the terminal portion of a tubule of the active proventriculus of a hen which had received an intravasculra injection of a "balanced" mixture of sodium ferrocyanide and ammonium ferric citrate. The production of hydrochloric acid demonstrated by the deposit of Prussian blue. x 1120

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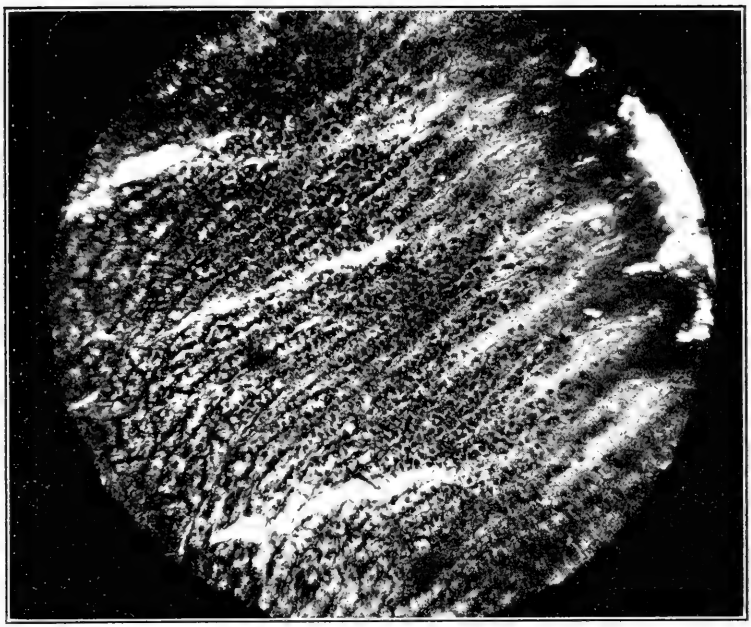

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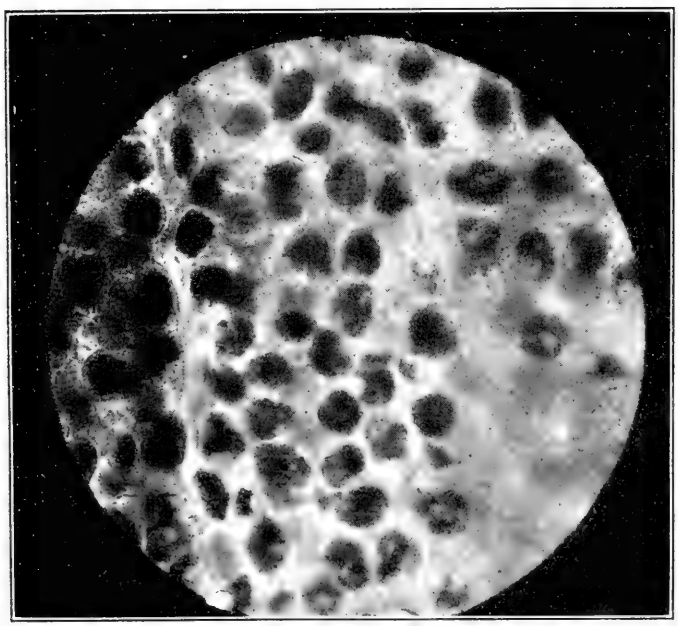

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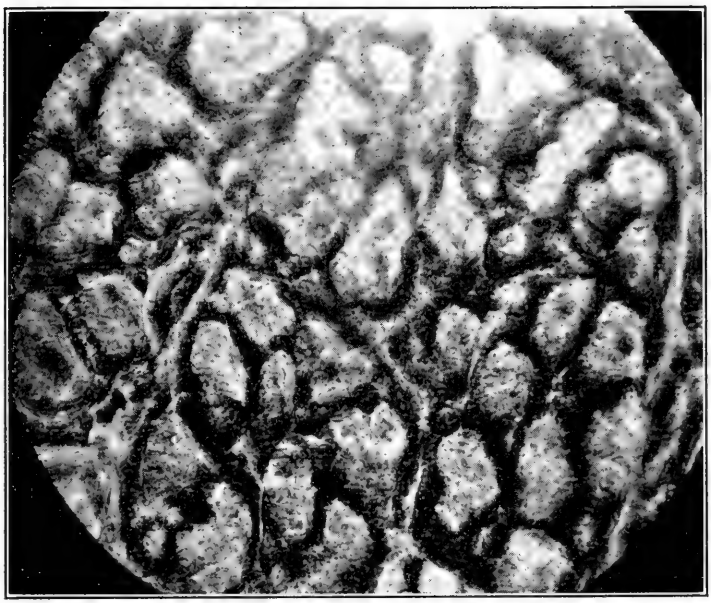

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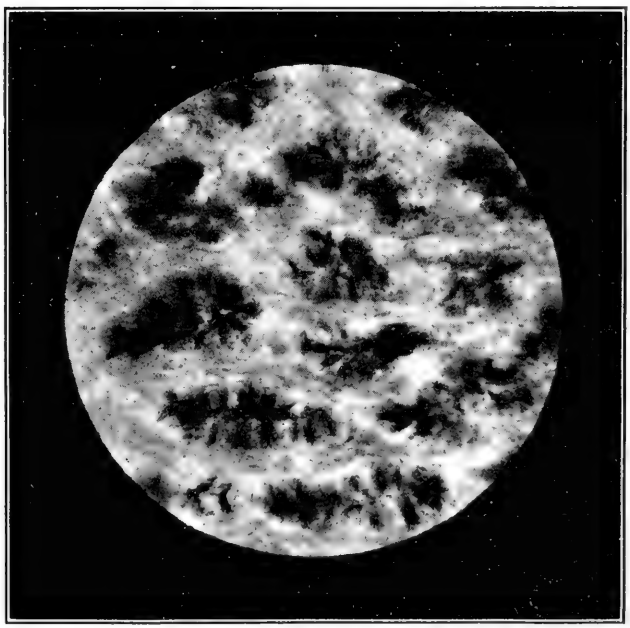

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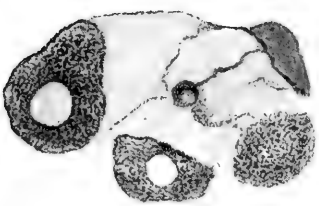
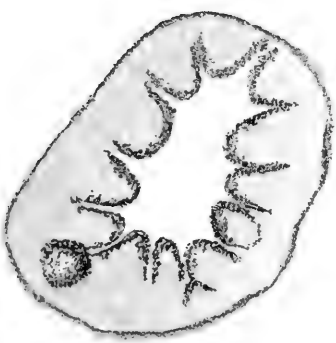


Drawing No. 1

Drawing No. 2



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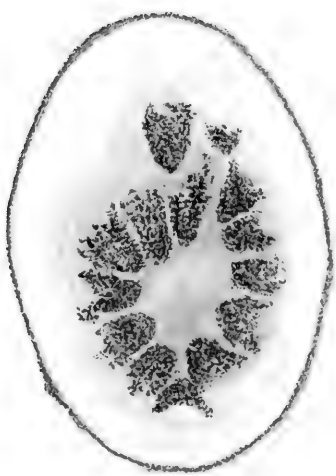

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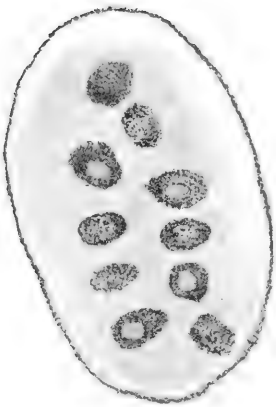

Drawing No. 5

Drawing No. 6

Drawing No. 7

Drawing No. 8

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