

TABLE I.  
Chemical Changes in the Developing Embryo of the Snapping Turtle.

| g                      | Egg  | Hatched turtle | Difference |
|------------------------|------|----------------|------------|
| Avg fresh wt           | 9.89 | 8.01           | —1.88      |
| " dry "                | 2.07 | 1.68           | —0.39      |
| " protein content      | 1.15 | 0.93           | —0.22      |
| " fat "                | 0.52 | 0.30           | —0.22      |
| " carbohydrate content | 0.14 | 0.16           | +0.02      |
| " inorganic "          | 0.17 | 0.19           | +0.02      |

ation.

Our results are summarized in Table I. It is obvious that the main substances consumed were fat and protein. Quantitatively, identical amounts of both groups of substances disappeared, but the greater amount of energy was obviously derived from the fat combustion. It will be noted that the sum of protein, fat, carbohydrate and inorganic substances amounts to about 95% of the dry weight in both batches. The 5% which is unaccounted for may be due to errors in the protein and carbohydrate determinations. It is questionable whether the customary conversion factor of 6.25 used in the protein determination is exactly applicable to turtle protein and it may well be that the carbohydrates present do not give exactly the same color intensity with the indole reagent as does the glucose solution used for comparison. The quantitative balance is sufficiently close, however, to prove that the developing snapper embryo derives its en-

ergy from the combustion of fat and protein. Similar observations have thus far been reported for only one other chelonian, the marine turtle *Thalassochelys corticata*.<sup>4,5</sup>

In our previous study<sup>1</sup> we found that a snapper egg consumes about 500 cc of oxygen during its development. The complete oxidation of 0.22 g of fat and 0.22 g of protein would require about 660 cc oxygen. If some carbohydrate were synthesized from protein, the figure would be slightly lower, but would still be in excess of 500 cc. The discrepancy may be explained by the fact that the eggs used this year were somewhat heavier than those employed 2 years ago (11.1 g as compared with 9.9 g) and that their development required an average of 75 days as against 72 days.

<sup>4</sup> Karashima, J., *J. Biochem. (Tokyo)*, 1929, **10**, 375.

<sup>5</sup> Nakamura, Y., *J. Biochem. (Tokyo)*, 1929, **10**, 357.

## 15700

### Effect of Anemic Anoxia on Absorption of Isotonic Magnesium Sulfate from the Small Intestine.\*

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It was shown by Northup and Van Liere<sup>1</sup> that even severe degrees of anoxic anoxia

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<sup>1</sup> Northup, D. W., and Van Liere, E. J., *Arch. Internat. Pharmac. et de Therap.*, 1939, **62**, 175.

<sup>2</sup> Van Liere, E. J., Northup, D. W., and Sleeth, C. K., *Am. J. Physiol.*, 1938, **124**, 102.

had no significant effect on absorption of magnesium sulfate from the small intestine of dogs. It had previously been shown,<sup>2</sup> however, that in anemic anoxia, produced by bleeding dogs 3.2% of their body weight, isotonic sodium chloride solution was absorbed from the small intestine more quickly than in control animals. The results were

TABLE I.  
Effect of Hemorrhage on the Absorption of Magnesium Sulfate.

| Exp. No. | Magnesium absorption |            | Sulfate absorption |            |
|----------|----------------------|------------|--------------------|------------|
|          | Control,<br>%        | Bled,<br>% | Control,<br>%      | Bled,<br>% |
| 1.       | —                    | 21         | —                  | 11         |
| 2.       | 15                   | 17         | 13                 | 17         |
| 3.       | 24                   | 17         | 24                 | 18         |
| 4.       | 17                   | 19         | 21                 | 17         |
| 5.       | 5                    | 26         | 10                 | 28         |
| 6.       | 16                   | 18         | 11                 | 18         |
| 7.       | 10                   | 17         | 9                  | 17         |
| 8.       | —                    | 9          | —                  | 7          |
| 9.       | 18                   | —          | 11                 | —          |
| 10.      | 5                    | 10         | 8                  | 14         |
|          | —                    | —          | —                  | —          |
| Avg      | 14                   | 17         | 13                 | 16*        |

\* None of the differences between the means are significant by Fisher's *t* test.

statistically significant.

was thought worthwhile to ascertain whether anemic anoxia (hemorrhage) would influence the rate of absorption of magnesium sulfate from the small intestine. It is known that this salt, if absorbed in sufficient quantities, may be quite toxic, so the problem is of practical as well as of academic interest.

**Methods.** Paired dogs as nearly alike in weight and age as possible were fasted 24 hours. One served as a control and the other was subjected to hemorrhage. Ten experiments were performed on a total of 20 animals.

The experimental animal was etherized, the femoral artery cannulated and blood withdrawn equal to 3% of the body weight. The artery was then ligated and the incision closed. Four hours were allowed for recovery from the ether and adjustment of the circulatory mechanism. During this interim, water was allowed *ad libitum*.

The animal was then given sodium barbital intravenously (300 mg/kg). The small intestine was exposed by a mid-line incision and practically the entire ileum used for a loop. The length of the loop was made the same as that in the control animal. It was washed out with isotonic glucose solution and filled with an isotonic solution of magnesium sulfate (3.25% anhydrous). After 90 minutes the fluid was removed from the loop and carefully measured. It was digested

with concentrated HCl and the amount of sulfate determined gravimetrically by precipitation with barium chloride. The magnesium was determined by precipitation with 8-hydroxyquinoline.

The control animal was treated exactly as the experimental with the important exception, of course, that it was not bled.

**Results.** Table I shows the results obtained. There was no great difference of absorption of either magnesium or sulfate between the controls and those subjected to hemorrhage. The controls absorbed 13% of the sulfate and 14% of the magnesium, while the experimental animals absorbed 16% and 17% respectively. The differences in the means of the results were not statistically significant. There was a close parallelism between the absorption of magnesium and of sulfate. In all cases, save one, the same amount or more of fluid was recovered than was put into the intestinal loop. In one experimental animal 13 cc less was recovered than was put in. The greatest amount of secretion was 11 cc.

**Discussion.** The results clearly indicate that a pronounced hemorrhage does not make the intestinal epithelium more permeable to either the magnesium or the sulfate ion.

As pointed out previously<sup>1</sup> it is of distinct interest that an appreciable amount of magnesium sulfate is absorbed from the small intestine, even in a period of 90 minutes. This is not generally appreciated, although

Hirschfelder<sup>3</sup> reported that after 20-40 g of magnesium sulfate were administered to a human subject, no increase occurred in the magnesium content of the blood, but 40% was excreted in the urine in 24 hours. How much of the sulfate was absorbed was not stated.

Verzar and McDougal<sup>4</sup> pointed out that the rate of absorption of the sulfate ion is about the same as that of substances of equal weight and compares favorably with that of pentoses and certain hexoses. The data we are presenting show a parallelism between the rates of absorption of the magnesium and the sulfate ions. So far as we are aware, this has not been previously emphasized.

Magnesium sulfate is widely employed by both practitioner and laymen and is probably one of the most commonly used household cathartics. A number of cases of magnesium poisoning<sup>5,6</sup> have rather recently been reported, some of which have proved fatal.

*Summary.* The rate of absorption of magnesium sulfate was studied in a series of barbitalized dogs each of which had suffered a hemorrhage equal to 3% of its body weight. The control animals absorbed 14% of the magnesium and 13% of the sulfate, whereas the experimental animals absorbed 17% and 16% respectively. The differences were not statistically significant. A fact not sufficiently appreciated is that the intestine is only relatively impermeable to this salt.

<sup>3</sup> Hirschfelder, A. D., *J. Biol. Chem.*, 1934, **104**, 647.

<sup>4</sup> Verzar, F., and McDougal, E. J., *Absorption from the Intestine*, Longmans, Green & Co., London, 1936.

<sup>5</sup> Bryon, F. E., *J. Malaya Br., Brit. M. A.*, 1939, **3**, 100.

<sup>6</sup> Gens, J. P., *J. A. M. A.*, 1943, **123**, 1028.

## 15701

### Estimation of Steroid Estrogens by Fluorimetry.\*

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Estrogens are still generally determined by biological methods. As these present all the difficulties and uncertainties of the biological technic, considerable interest attaches to the development of an adequate chemical assay for the estrogens. During more than a decade repeated attempts have been made to elaborate a colorimetric procedure suitable to this purpose.<sup>1-10</sup> Most of this work has been based on the Kober reaction. The

available colorimetric methods are, however, difficult in practice, of imperfect specificity, and often insufficient in sensitivity. A search in the field of fluorimetry for an adequate solution of this analytical problem would seem to be indicated. The proven high sensitivity of fluorimetric methods in vitamin assay encourages the hope that methods of this type could find usefulness in determination of hormones at biological level.

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<sup>1</sup> Kober, S., *Biochem. Z.*, 1931, **239**, 209.

<sup>2</sup> Kober, S., *Biochem. J.*, 1938, **32**, 357.

<sup>3</sup> Cartland, F. G., Meyer, R. K., Miller, L. C., and Rutz, M. H., *J. Biol. Chem.*, 1935, **109**, 213.

<sup>4</sup> Venning, E. H., Evelyn, K. A., Harkness, E. V., and Browne, Y. S. L., *J. Biol. Chem.*, 1937, **120**, 225.

<sup>5</sup> Baehman, C., *J. Biol. Chem.*, 1939, **131**, 455.

<sup>6</sup> Bachman, C., and Pettit, D. S., *J. Biol. Chem.*, 1941, **138**, 689.

<sup>7</sup> Talbot, N. B., Wolfe, Y. K., MacLaehlan, E. A., Karush, F., and Butler, A. M., *J. Biol. Chem.*, 1940, **134**, 319.

<sup>8</sup> David, K., *Acta brev. Neerland*, 1934, **4**, 64.

<sup>9</sup> Zimmermann, W., *Z. physiol. Chem.*, 1935, **233**, 257; 1936-37, **245**, 47.

<sup>10</sup> Pineus, G., Wheeler, G., Young, G., and Zahl, P. A., *J. Biol. Chem.*, 1936, **116**, 253.