

# EXPERIMENTAL STUDIES ON FAT METABOLISM FROM THE VIEW-POINT OF KETONE BODY FORMATION

by

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We have succeeded in the production of a fat emulsion, which consists principally of neutral fat and can be administered intravenously.<sup>36)</sup> ASADA,<sup>3)</sup> NAKATA,<sup>67)</sup> NISHINO<sup>70)</sup> and SENŌ<sup>82)</sup> examined histo- and biochemically the metabolic process of the fat emulsion in our laboratory and demonstrated clearly that; the infused fat globules were first phagocytized by the cells of the reticuloendothelial system in the lung, liver and spleen. The fatty acids, contained in the infused fat emulsion, were oxidized after neutral fat was changed into phospholipid in these cells.

Studies on fatty acid oxidation retarded in contrast to the advances in the field of carbohydrate metabolism. More rapid progress has been made since MUÑOZ and LELOIR<sup>56,57,66)</sup> applied washed liver particles in oxidation of short chain fatty acids. Studies on long chain fatty acids, however, have been hardly performed and their metabolism is still not completely understood. The outline of fat metabolism, which is generally recognized now, is considered as Fig. 1 : Fatty acid is converted completely to 2-carbon fragments viz. acetyl coenzyme A (acetyl-CoA) by successive  $\beta$ -oxidation. A part of acetyl-CoA enters into the tricarboxylic acid cycle (T.C.A. cycle) and pairs of the part react together to form acetoacetic acid (ketone body), which diffuses into the blood stream and is carried to the extrahepatic tissues, especially muscle and kidney etc.. After the ketone body converts again into acetyl-CoA and is activated in these tissues, acetyl-CoA enters indirectly into the T.C.A. cycle, in which it is oxidized completely to carbon dioxide and water<sup>1,8,13 14 16, 30, 46, 49, 51, 52, 59, 60, 102)</sup>. All enzyme systems catalizing the reactions of fatty acid oxidation are contained in mitochondria.

If the changes of blood and urine ketone bodies levels following intravenous infusion of the fat emulsion are investigated, we shall be able to judge whether the fat emulsion is utilized effectively and smoothly, and to decide the methods, by which the administration of the fat emulsion can be most effectively done for purpose of parenteral nutrition.

## MATERIALS AND METHODS

*Experimental Animals* — Healthy male albino rabbits, each weighting approximately 2 kg, were used. They had been fed by the standard diet for two weeks and were fasted for 24 hours, being in the post-absorptive state, at the onset of the experiments. In case of necessity, adult dogs or cats were used.

*Collection of Urine Specimens* — The bladder was completely emptied by



catheterization before beginning of the experiments. After intravenous administration of the fat emulsion, the rabbits were kept in cages with urine collecting apparatus. After 24 hours the rabbits were catheterized again. The sum of the accumulated urine in the apparatus and the catheterized urine was regarded as the 24 hour urine specimen.

*Fat Emulsion* — In the present investigation, 15 per cent sesame oil emulsion (Emulsion A) was used as a rule. This fat emulsion contains 15 per cent sesame oil, 7 per cent glucose and small quantities of stabilizers. Its principal fat component is neutral fat. In case of necessity, 15 per cent sesame oil emulsion containing no glucose (Emulsion B) or 15 per cent cod liver oil emulsion (Emulsion C) was used. The average daily ingestion of fat by Japanese people is 0.5 g per kg of body weight, as is widely known. The standard dose of the intravenous administration of the fat emulsion was defined as 3.3 cc of 15 per cent fat emulsion, which is equal to 0.5 g of fat, per kg of body weight.

*Used Drugs* — In the present investigation, methionine as l-methionine, riboflavin as riboflavin-5'-phosphate, vitamin C as l-ascorbic acid, nicotinic acid as niacin amide, pantothenic acid as calcium pantothenate and vitamin B<sub>1</sub> as thiamin hydrochloride, were used in the formation of the solution.

*Procedures of Measurement* — The determination of ketone bodies was made by the method of GREENBERG and LESTER<sup>38)</sup> and the total ketone bodies were expressed as acetone. Blood sugar levels were measured by the method of SOMOGYI.<sup>40)</sup>

## RESULTS

### 1. *Perfusion Experiment of the Isolated Liver*

As above mentioned, ASADA,<sup>5)</sup> IZUKURA,<sup>40)</sup> NAKATA<sup>67)</sup> and SENŌ<sup>8,1)</sup> established histo- and biochemically that the infused fat globules were phagocytized by the cells of the reticuloendothelial system and entered into the hepatic parenchymatous cells after lipoidization of neutral fat by the former cells. The present perfusion experiment of the isolated liver, furthermore, ascertained biochemically how the fat emulsion, which entered into the hepatic parenchymatous cells in the form of phospholipid, was metabolized. Its techniques and conditions followed those of SENŌ.<sup>8,2)</sup> The isolated livers were perfused through the portal vein for two hours with CHASHI's closed system circulator. Defibrinated blood of the same individuals, whose livers were isolated, was diluted three times with modified RINGER's solution, and 150 cc was used as the circulating fluid (Basic fluid).

*Perfusion of Isolated Rabbit Liver* — As shown in Table I, ketone bodies levels in the circulating fluid in the perfusion with the basic fluid plus Emulsion A, 1.2 cc increased in contrast to the controls with the basic fluid alone. Perfusions were made with the addition of methionine, 14 mg and vitamin B<sub>12</sub>, 2γ, both of which accelerated lipoidization of the fat emulsion,<sup>5, 67, 82)</sup> to the basic fluid plus Emulsion A, 1.2 cc. The results in Table II showed that ketone bodies levels in the circulating fluid increased slightly in contrast to the perfusions with the basic fluid plus Emulsion A, 1.2 cc.

**Table I.** *Ketone Bodies Levels in the Circulating Fluid of the Perfused Rabbit Liver.*  
(Emulsion A, 1.2cc was added to the basic fluid.)

| Remarks                                | No. of rabbit | Weight of liver | Time of perfusion (min.) |               |               |               |               |               | Increase      | Difference from control |
|----------------------------------------|---------------|-----------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------------|
|                                        |               |                 | 0                        | 15            | 30            | 60            | 90            | 120           |               |                         |
| Basic fluid                            | 1             | g<br>62         | mg/dl<br>0.25            | mg/dl<br>0.46 | mg/dl<br>0.59 | mg/dl<br>0.96 | mg/dl<br>1.19 | mg/dl<br>1.64 | mg/dl<br>1.39 | 0                       |
|                                        | 2             | 58              | 0.20                     | 0.21          | 0.60          | 0.93          | 1.13          | 1.39          | 1.19          |                         |
|                                        | 5             | 70              | 0.10                     | 0.21          | 0.21          | 0.46          | 0.69          | 0.86          | 0.76          |                         |
|                                        | Mean          | 63              | 0.18                     | 0.29          | 0.47          | 0.78          | 1.00          | 1.30          | 1.12          |                         |
|                                        |               |                 |                          |               |               |               |               |               |               |                         |
| Basic fluid +<br>Emulsion A<br>(1.2cc) | 6             | 67              | 0.21                     | 0.46          | 1.17          | 1.33          | 1.69          | 2.40          | 2.19          | 1.51                    |
|                                        | 8             | 60              | 0.10                     | 0.21          | 0.96          | 1.48          | 2.14          | 3.29          | 3.19          |                         |
|                                        | 9             | 68              | 0.10                     | 0.21          | 0.70          | 1.19          | 2.12          | 2.63          | 2.53          |                         |
|                                        | Mean          | 65              | 0.14                     | 0.29          | 0.94          | 1.33          | 1.98          | 2.77          | 2.63          |                         |
|                                        |               |                 |                          |               |               |               |               |               |               |                         |

**Table II.** *Ketone Bodies Levels in the Circulating Fluid of the Perfused Rabbit Liver.*  
(Emulsion A, 1.2cc, methionine, 14mg and vitamin B<sub>12</sub>, 2γ were added to the basic fluid.)

| Remarks                                                             | No. of rabbit | Weight of liver | Time of perfusion (min.) |               |               |               |               |               | Increase      | Difference from control |
|---------------------------------------------------------------------|---------------|-----------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------------|
|                                                                     |               |                 | 0                        | 15            | 30            | 60            | 90            | 120           |               |                         |
| Basic fluid +<br>Methionine +<br>V. B <sub>12</sub>                 | 10            | g<br>57         | mg/dl<br>0.10            | mg/dl<br>0.10 | mg/dl<br>0.36 | mg/dl<br>0.69 | mg/dl<br>1.29 | mg/dl<br>1.62 | mg/dl<br>1.52 | 0                       |
|                                                                     | 11            | 61              | 0.10                     | 0.21          | 0.48          | 1.39          | 1.62          | 1.96          | 1.86          |                         |
|                                                                     | 13            | 58              | 0.46                     | 0.83          | 0.94          | 1.29          | 1.65          | 2.00          | 1.54          |                         |
|                                                                     | Mean          | 59              | 0.22                     | 0.38          | 0.59          | 1.12          | 1.52          | 1.86          | 1.64          |                         |
|                                                                     |               |                 |                          |               |               |               |               |               |               |                         |
| Basic fluid +<br>Emulsion A +<br>Methionine +<br>V. B <sub>12</sub> | 15            | 58              | 0.10                     | 0.46          | 0.96          | 1.55          | 2.20          | 3.30          | 3.20          | 1.91                    |
|                                                                     | 16            | 72              | 0.10                     | 0.25          | 0.70          | 1.20          | 3.53          | 3.90          | 3.80          |                         |
|                                                                     | 17            | 55              | 0.10                     | 0.21          | 0.46          | 1.12          | 2.67          | 3.74          | 3.64          |                         |
|                                                                     | Mean          | 62              | 0.10                     | 0.31          | 0.71          | 1.29          | 2.80          | 3.65          | 3.55          |                         |
|                                                                     |               |                 |                          |               |               |               |               |               |               |                         |

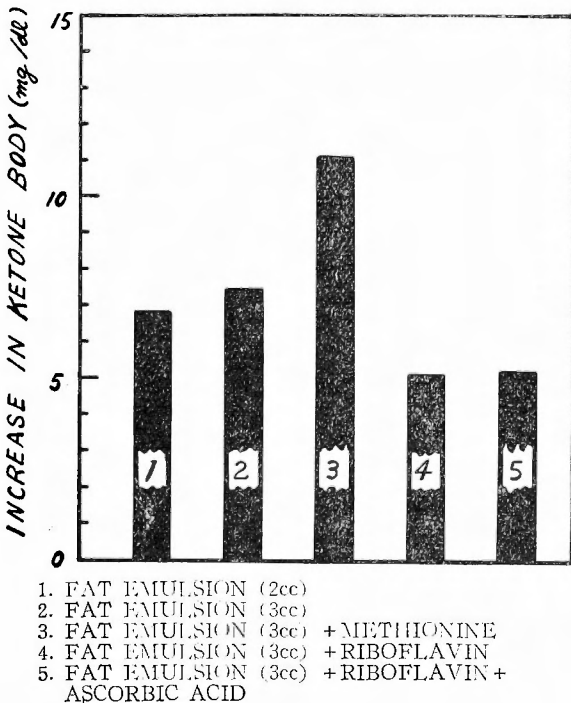
*Perfusion of isolated Cat Liver* — Isolated livers from adult cats, ranging in weight from 2.5 kg to 3.0 kg, were perfused as well as rabbits. Perfusions with the basic fluid plus Emulsion A, 2 cc or 3 cc showed the results in Table III that increase in ketone bodies levels in the circulating fluid was more remarkable than the controls with the basic fluid alone. Slight increase in ketone bodies levels was observed even in the controls probably by reason of the fact that fatty acids, which were contained physiologically in the liver and blood, were oxidized.

From these results and previous reports from our laboratory,<sup>5, 10, 82)</sup> we are able to postulate that the fat globules added to the circulating fluid were phagocytized by KUPPER's cells, in which they were changed into phospholipid and thereafter a part of the fatty acids contained in the infused fat emulsion entered into the T.C.A. cycle via the stage of acetyl-CoA. Pairs of the surplus acetyl-CoA react together to form acetoacetic acid which diffuses into the circulating fluid. Accordingly these results in the present perfusion experiment indicate that the fat emulsion can be

**Table III.** *Ketone Bodies Levels in the Circulating Fluid of the Perfused Cat Liver.*  
(Emulsion A, 2cc or 3cc was added to the basic fluid.)

| Remarks                         | No. of cat | Weight of liver | Time of perfusion (min.) |            |            |            |            |            | Increase   | Difference from control |
|---------------------------------|------------|-----------------|--------------------------|------------|------------|------------|------------|------------|------------|-------------------------|
|                                 |            |                 | 0                        | 15         | 30         | 60         | 90         | 120        |            |                         |
| Basic fluid                     | 1          | g 95            | mg/dl 0.46               | mg/dl 0.57 | mg/dl 0.93 | mg/dl 0.93 | mg/dl 1.12 | mg/dl 1.38 | mg/dl 0.92 | 0                       |
|                                 | 2          | 130             | 0.10                     | 0.10       | 0.46       | 0.66       | 0.92       | 1.38       | 1.28       |                         |
|                                 | 3          | 130             | 0.36                     | 0.56       | 0.56       | 1.38       | 1.94       | 2.10       | 1.74       |                         |
|                                 | 5          | 87              | 0.46                     | 0.92       | 1.53       | 1.63       | 2.10       | 2.75       | 2.29       |                         |
|                                 | 7          | 118             | 0.36                     | 0.55       | 0.59       | 0.70       | 0.86       | 1.36       | 1.00       |                         |
|                                 | Mean       | 112             | 0.35                     | 0.61       | 0.85       | 1.06       | 1.39       | 1.79       | 1.44       |                         |
| Basic fluid + Emulsion A (2 cc) | 8          | 90              | 0.46                     | 1.38       | 2.50       | 4.13       | 7.31       | 8.57       | 8.11       | 6.79                    |
|                                 | 10         | 90              | 0.46                     | 1.12       | 2.60       | 4.38       | 6.37       | 8.76       | 8.30       |                         |
|                                 | 11         | 89              | 0.46                     | 1.12       | 2.70       | 1.13       | 5.51       | 8.75       | 8.29       |                         |
|                                 | Mean       | 90              | 0.46                     | 1.21       | 2.60       | 4.21       | 6.40       | 8.69       | 8.23       |                         |
| Basic fluid + Emulsion A (3 cc) | 12         | 102             | 0.20                     | 1.27       | 1.99       | 5.20       | 5.82       | 7.60       | 7.40       | 7.39                    |
|                                 | 13         | 117             | 0.10                     | 1.12       | 2.64       | 4.91       | 6.37       | 10.45      | 10.35      |                         |
|                                 | 14         | 106             | 0.36                     | 1.12       | 2.50       | 5.96       | 6.12       | 9.10       | 8.74       |                         |
|                                 | Mean       | 108             | 0.22                     | 1.17       | 2.38       | 5.36       | 6.10       | 9.05       | 8.83       |                         |

**Fig. 2.** Influence of various drugs on ketone body formation from the fat emulsion by perfused cat liver.



oxidized by the hepatic parenchymatous cells. As compared with the results in the cat and rabbit, the former viz. carnivore is stronger in the ability to dispose of fat than the latter viz. herbivore.

The results in Table IV and Fig. 2 were obtained by perfusions with the addition of methionine, 14 mg and vitamin B<sub>12</sub>, 2 γ to the basic fluid plus Emulsion A, 3 cc. Ketone bodies levels in the circulating fluid increased more significantly than those in the methionine controls or in perfusions with the basic fluid plus Emulsion A, 3 cc (Table IV and Fig. 2). Since methionine is not a ketogenic amino acid,<sup>8,18,74)</sup> it is accepted, as mentioned by Senō<sup>82)</sup>, that methionine and vitamin B<sub>12</sub> accelerate lipoidization of neutral fat by KUPPER's cells and secondarily

**Table IV.** *Ketone Bodies Levels in the Circulating Fluid of the Perfused Cat Liver.*  
(Emulsion A, 3cc, methionine, 14mg and vitamin B<sub>12</sub>, 2γ were added to the basic fluid.)

| Remarks                                                             | No. of cat | Weight of liver | Time of perfusion (min.) |               |               |               |               |               | Increase      | Difference from control |
|---------------------------------------------------------------------|------------|-----------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------------|
|                                                                     |            |                 | 0                        | 15            | 30            | 60            | 90            | 120           |               |                         |
| Basic fluid +<br>Methionine +<br>V. B <sub>12</sub>                 | 15         | g<br>102        | mg/dl<br>0.20            | mg/dl<br>1.38 | mg/dl<br>1.74 | mg/dl<br>1.84 | mg/dl<br>2.09 | mg/dl<br>3.88 | mg/dl<br>3.68 | 0                       |
|                                                                     | 16         | 89              | 0.20                     | 1.74          | 2.09          | 3.11          | 3.37          | 3.62          | 3.42          |                         |
|                                                                     | 17         | 120             | 0.54                     | 0.99          | 1.26          | 1.77          | 2.86          | 3.07          | 2.53          |                         |
|                                                                     | Mean       | 104             | 0.31                     | 1.37          | 1.70          | 2.24          | 2.77          | 3.52          | 3.21          |                         |
| Basic fluid +<br>Emulsion A +<br>Methionine +<br>V. B <sub>12</sub> | 18         | 97              | 0.36                     | 0.93          | 2.86          | 6.53          | 10.01         | 11.66         | 11.30         | 11.06                   |
|                                                                     | 19         | 95              | 0.46                     | 1.27          | 3.10          | 10.53         | 12.44         | 16.26         | 15.80         |                         |
|                                                                     | 28         | 97              | 0.10                     | 1.27          | 3.36          | 8.87          | 11.81         | 15.81         | 15.71         |                         |
|                                                                     | Mean       | 96              | 0.31                     | 1.16          | 3.11          | 8.64          | 11.42         | 14.58         | 14.27         |                         |

**Table V.** *Ketone Bodies Levels in the Circulating Fluid of the Perfused Cat Liver.*  
(Emulsion A, 3cc and riboflavin, 5mg were added to the basic fluid.)

| Remarks                                     | No. of cat | Weight of liver | Time of perfusion (min.) |               |               |               |               |               | Increase      | Difference from control |
|---------------------------------------------|------------|-----------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------------|
|                                             |            |                 | 0                        | 15            | 30            | 60            | 90            | 120           |               |                         |
| Basic fluid +<br>Riboflavin                 | 21         | g<br>84         | mg/dl<br>0.10            | mg/dl<br>0.19 | mg/dl<br>0.19 | mg/dl<br>1.12 | mg/dl<br>2.29 | mg/dl<br>2.45 | mg/dl<br>2.35 | 0                       |
|                                             | 22         | 128             | 0.19                     | 0.19          | 0.56          | 1.91          | 1.98          | 2.29          | 2.10          |                         |
|                                             | 23         | 80              | 0.45                     | 0.62          | 1.58          | 2.15          | 2.71          | 2.91          | 2.46          |                         |
|                                             | Mean       | 97              | 0.25                     | 0.33          | 0.78          | 1.73          | 2.33          | 2.55          | 2.30          |                         |
| Basic fluid +<br>Emulsion A +<br>Riboflavin | 25         | 119             | 0.68                     | 1.31          | 2.41          | 3.79          | 5.40          | 7.75          | 7.07          | 5.07                    |
|                                             | 27         | 127             | 0.68                     | 1.17          | 2.17          | 3.59          | 6.78          | 7.66          | 6.98          |                         |
|                                             | 30         | 86              | 0.64                     | 1.41          | 2.44          | 5.94          | 6.72          | 8.72          | 8.08          |                         |
|                                             | Mean       | 111             | 0.67                     | 1.30          | 2.34          | 4.44          | 6.30          | 8.04          | 7.37          |                         |

**Table VI.** *Ketone Bodies Levels in the Circulating Fluid of the Perfused Cat Liver.*  
(Emulsion A, 3cc, riboflavin, 5mg and ascorbic acid, 25mg were added to the basic fluid.)

| Remarks                                                        | No. of cat | Weight of liver | Time of perfusion (min.) |               |               |               |               |               | Increase      | Difference from control |
|----------------------------------------------------------------|------------|-----------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------------|
|                                                                |            |                 | 0                        | 15            | 30            | 60            | 90            | 120           |               |                         |
| Basic fluid +<br>Riboflavin +<br>Ascorbic acid                 | 32         | g<br>121        | mg/dl<br>0.64            | mg/dl<br>1.10 | mg/dl<br>1.67 | mg/dl<br>2.41 | mg/dl<br>2.85 | mg/dl<br>3.69 | mg/dl<br>3.05 | 0                       |
|                                                                | 34         | 85              | 0.64                     | 1.45          | 2.00          | 2.60          | 3.11          | 3.35          | 2.71          |                         |
|                                                                | 35         | 119             | 0.45                     | 1.55          | 2.60          | 2.85          | 2.86          | 3.17          | 2.72          |                         |
|                                                                | Mean       | 108             | 0.58                     | 1.37          | 2.09          | 2.62          | 2.94          | 3.40          | 2.82          |                         |
| Basic fluid +<br>Emulsion A +<br>Riboflavin +<br>Ascorbic acid | 37         | 86              | 0.45                     | 0.95          | 1.86          | 4.24          | 6.93          | 8.31          | 7.86          | 5.22                    |
|                                                                | 38         | 95              | 0.73                     | 1.77          | 3.18          | 5.68          | 8.60          | 9.62          | 8.89          |                         |
|                                                                | 41         | 90              | 0.68                     | 1.67          | 2.41          | 3.73          | 6.83          | 8.06          | 7.38          |                         |
|                                                                | Mean       | 90              | 0.62                     | 1.46          | 2.48          | 4.55          | 7.45          | 8.66          | 8.04          |                         |

Perfusions with the addition of riboflavin, 5 mg to the basic fluid plus Emulsion A, 3c.c. , or with the addition of riboflavin, 5 mg and ascorbic acid, 25 mg to the basic fluid plus Emulsion A, 3 cc were made. As shown in Table V, VI and Fig. 2, increase in ketone bodies levels in the circulating fluid was slightly less than in perfusions with the basic fluid plus Emulsion A, 3 cc alone. These results indicate that the liver weakly utilizes ketone bodies<sup>65, 68)</sup> and that its action is intensified to some degree by the addition of these enzyme systems, which are concerned in fat metabolism.

When the standard dose of Emulsion A was administered intravenously into rabbits, blood ketone bodies began to increase 6 hours after infusion and reached its maximal level 12 hours after infusion. Thereafter the level decreased and were

| Remarks                               | No. of rabbit | Blood      |            |            |            |            |            |            |            |            |               | Urine     |  |
|---------------------------------------|---------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|---------------|-----------|--|
|                                       |               | 0          | 1hrs.      | 3hrs.      | 6hrs.      | 9hrs.      | 12hrs.     | 18hrs.     | 24hrs.     | 30hrs.     | Concentration | Excretion |  |
| Control (7 per cent glucose solution) | 18            | mg/dl 0.92 | mg/dl 0.92 | mg/dl 1.12 | mg/dl 1.38 | mg/dl 0.82 | mg/dl 0.82 | mg/dl 1.02 | mg/dl 1.12 | mg/dl 1.12 | mg/dl 1.72    | mg 1.20   |  |
|                                       | 21            | 0.56       | 0.66       | 0.66       | 1.28       | 0.66       | 1.38       | 1.38       | 0.46       | 1.02       | 1.76          | 1.41      |  |
|                                       | 22            | 0.46       | 0.66       | 0.82       | 1.28       | 1.38       | 1.38       | 0.56       | 0.92       | 1.12       | 1.14          | 1.14      |  |
|                                       | 25            | 0.84       | 0.85       | 1.53       | 0.05       | 1.44       | 0.46       | 1.33       | 1.67       | 1.33       | 1.17          | 1.40      |  |
|                                       | Mean          | 0.70       | 0.77       | 1.03       | 1.20       | 1.08       | 1.01       | 1.07       | 1.04       | 1.15       | 1.45          | 1.29      |  |
|                                       | Change(%)     | 0          | + 10       | + 47       | + 71       | + 54       | + 44       | + 53       | + 49       | + 64       |               |           |  |
| Emulsion A (3.3cc per kg.)            | 20            | 0.96       | 1.01       | 1.16       | 1.73       | 1.45       | 2.60       | 1.92       | 1.61       | 2.33       | 2.04          | 3.47      |  |
|                                       | 23            | 0.35       | 0.44       | 0.63       | 1.20       | 1.39       | 2.91       | 2.33       | 0.94       | 2.12       | 1.85          | 2.78      |  |
|                                       | 27            | 0.56       | 0.72       | 0.91       | 1.30       | 1.74       | 2.64       | 2.20       | 1.71       | 2.03       | 1.72          | 2.41      |  |
|                                       | 28            | 0.65       | 0.84       | 1.02       | 1.07       | 1.98       | 2.52       | 1.94       | 1.72       | 3.88       | 1.86          | 2.79      |  |
|                                       | Mean          | 0.63       | 0.75       | 0.93       | 1.33       | 1.64       | 2.67       | 2.10       | 1.50       | 2.59       | 1.87          | 2.86      |  |
|                                       | Change(%)     | 0          | + 19       | + 48       | + 111      | + 160      | + 324      | + 233      | + 138      | + 311      |               |           |  |

**Table VIII.** *Ketone Bodies Levels Following Intravenous Infusion of Emulsion A or Emulsion C.*

| Remarks                       | No. of rabbit | Blood         |               |               |               |               |               |               |               |               |                | Urine      |  |
|-------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|------------|--|
|                               |               | 0             | 1hrs.         | 3hrs.         | 6hrs.         | 9hrs.         | 12hrs.        | 18hrs.        | 24hrs.        | 30hrs.        | Concent-ration | Excre-tion |  |
| Emulsion A<br>(1.7cc per kg.) | 29            | mg/dl<br>0.36 | mg/dl<br>0.36 | mg/dl<br>1.04 | mg/dl<br>1.96 | mg/dl<br>1.72 | mg/dl<br>1.72 | mg/dl<br>1.57 | mg/dl<br>1.62 | mg/dl<br>1.85 | mg/dl<br>1.83  | mg<br>2.20 |  |
|                               | 30            | 1.15          | 0.92          | 1.62          | 2.11          | 1.07          | 1.68          | 1.53          | 1.75          | 1.56          | 1.30           | 1.69       |  |
|                               | 33            | 0.92          | 0.92          | 1.02          | 1.45          | 1.33          | 1.31          | 1.62          | 1.30          | 1.53          | 1.52           | 1.52       |  |
|                               | 36            | 0.44          | 1.10          | 1.36          | 1.42          | 1.88          | 1.71          | 1.80          | 2.33          | 1.39          | 2.83           | 2.26       |  |
|                               | Mean          | 0.72          | 0.83          | 1.26          | 1.74          | 1.50          | 1.61          | 1.63          | 1.75          | 1.58          | 1.87           | 1.92       |  |
|                               | Change(%)     | 0             | +15           | +75           | +142          | +108          | +124          | +126          | +143          | +119          |                |            |  |
| Emulsion A<br>(6.6cc per kg.) | 31            | 0.78          | 1.33          | 1.93          | 1.92          | 2.53          | 3.10          | 2.90          | 2.58          | 3.03          | 3.40           | 3.40       |  |
|                               | 34            | 0.86          | 1.35          | 1.93          | 2.11          | 2.62          | 2.04          | 2.09          | 1.89          | 1.40          | 2.58           | 3.10       |  |
|                               | 35            | 0.92          | 1.14          | 1.72          | 1.95          | 2.73          | 2.08          | 2.29          | 3.31          | 2.42          | 3.94           | 3.94       |  |
|                               | 37            | 0.46          | 1.16          | 2.37          | 2.59          | 1.77          | 1.96          | 1.48          | 1.68          | 2.23          | 2.37           | 2.84       |  |
|                               | Mean          | 0.76          | 1.25          | 1.99          | 2.14          | 2.41          | 2.30          | 2.19          | 2.37          | 2.27          | 3.07           | 3.32       |  |
|                               | Change(%)     | 0             | +64           | +162          | +182          | +217          | +203          | +188          | +212          | +199          |                |            |  |
| Emulsion C<br>(3.3cc per kg.) | 38            | 0.73          | 1.80          | 2.02          | 4.15          | 1.12          | 1.52          | 2.00          | 2.60          | 1.52          |                |            |  |
|                               | 39            | 0.51          | 1.01          | 1.01          | 1.80          | 0.73          | 2.76          | 2.00          | 2.87          | 1.13          |                |            |  |
|                               | 40            | 0.57          | 1.12          | 1.66          | 2.02          | 2.39          | 1.34          | 1.84          | 2.38          | 1.40          |                |            |  |
|                               | 42            | 0.67          | 1.12          | 1.53          | 2.72          | 2.00          | 1.12          | 1.84          | 2.10          | 1.38          |                |            |  |
|                               | Mean          | 0.62          | 1.26          | 1.56          | 2.67          | 1.56          | 1.69          | 1.92          | 2.49          | 1.36          |                |            |  |
|                               | Change(%)     | 0             | +103          | +152          | +331          | +152          | +173          | +210          | +302          | +119          |                |            |  |

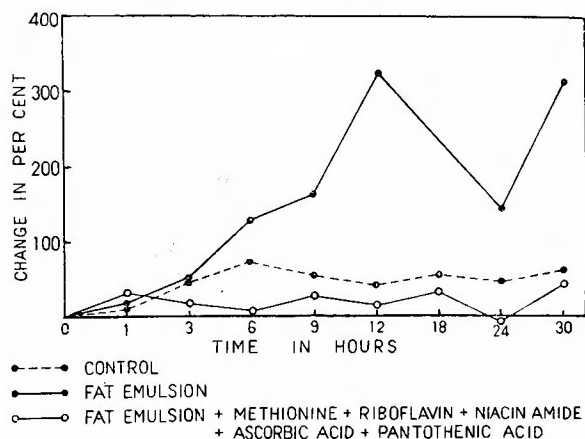
slightly higher than the controls 24 hours after infusion. The urinary ketone bodies excretion was far more than that in the controls (Table VII and Fig. 3). Following the intravenous administration of a half or twice of the standard dose, blood and urine ketone bodies levels fluctuated correspondingly without abnormal deviation (Table VIII).

Instead of Emulsion A, an equivalent dose of Emulsion C was administered intravenously. The results are shown in Table VIII. Blood ketone bodies levels increased early after infusion, with marked fluctuations, and showed as high as three times of the pre-experimental levels 24 hours after infusion. KENNEDY and LEHNINGER<sup>46)</sup> found that short chain even carbon fatty acids formed chiefly acetoacetic acid in the liver, while long chain even carbon fatty acids yielded chiefly carbon dioxide under identical conditions. Short chain fatty acids are obligatory ketogenic, and long chain fatty acids are facultative ketogenic.<sup>61, 74, 102)</sup> Since cod liver oil contains large quantities of short chain fatty acids in contrast to sesame oil, the above results would be obtained. It should be also considered that short chain fatty acids are directly oxidized in the tissues while long chain fatty acids are able to stored in fat depots.<sup>74, 78, 81, 82)</sup>

*Simultaneous Infusion of Various Drugs with the Fat Emulsion* — Previous reports from our laboratory<sup>5, 67, 82)</sup> and the present perfusion experiment demonstrated

that methionine accelerated phagocytosis and lipidization of the fat emulsion by the reticuloendothelial cells and secondarily expedited fatty acid oxidation in the hepatic parenchymatous cells. Furthermore, TSUKADA,<sup>96)</sup> OSA,<sup>73)</sup> NISHINO<sup>70)</sup> and TAKEDA<sup>91)</sup> reported that the intravenous administration of the fat emulsion was made nutritiously effective by the simultaneous administration of riboflavin. After phosphorylation in the intestinal mucosa, riboflavin synthesizes flavin adenine dinucleotide (F.A.D.) chiefly in the kidney and liver<sup>105)</sup>. Riboflavin is biochemically active only in the form of F.A.D. or flavin mononucleotide (F.M.N.). *In vivo* the major part of riboflavin is present in the form of F.A.D., while F.M.N. is found in small quantities and free riboflavin is the least quantity.<sup>25, 125)</sup> Therefore it is expedient that F.A.D. or at least F.M.N. is infused simultaneously in the case of intravenous administration of the fat emulsion, but at present pure F.A.D. is expensive to produce. Since riboflavin-5'-phosphate synthesized by FLEXSNER and FARKAS<sup>24)</sup> is identical to F.M.N., riboflavin-5'-phosphate sodium solution was used in the present investigation. Recently NATH and CHAKRABARTY<sup>60)</sup> reported that rabbits receiving continuous injection of sodium acetoacetate and  $\beta$ -hydroxybutyrate for three months showed a state of riboflavin and nicotinic acid deficiency. HIKASA and ISHIGAMI<sup>36-a)</sup> observed that simultaneous infusion of ascorbic acid with the fat emulsion was effective in aiding metabolism of the infused fat emulsion as a result of intensification of activity of lipase in serum and the liver. In fatty acid oxidation, as above mentioned, fatty acid is oxidized in the form of derivatives of coenzyme A by successive  $\beta$ -oxidation, and consequently is converted to acetyl-CoA,<sup>1, 9, 17, 58, 60, 63)</sup> which enters into the T.C.A. cycle by condensation with oxaloacetic acid. It has been established that in turn of T.C.A. cycle  $\alpha$ -ketoglutaric acid converts to succinic acid via succinyl-CoA by combination with coenzyme A and by oxidative decarboxylation<sup>26, 45, 79)</sup>. Pairs of the surplus acetyl-CoA, unable to enter into the T.C.A. cycle, react together to form acetoacetic acid (ketone body), which diffuses into the blood stream and is carried to the extrahepatic tissues. In these tissues ketone bodies convert

**Fig. 3.** Changes in blood ketone bodies levels following intravenous infusion of the fat emulsion.



again into acetyl-CoA and indirectly enter into the T.C.A. cycle.<sup>32, 54, 63, 91, 92)</sup> Therefore coenzyme A plays a very important role in fat metabolism. It is evident from the studies of LIPMANN and his co-workers<sup>52, 72)</sup> that pantothenic acid is a chemical component of coenzyme A.

According to the above mentioned reports, the author investigated how the metabolic process of the fat emulsion was improved by the simultaneous infusion of the following drugs. Methionine, 5 mg,

**Table IX.** *Effect of Simultaneous Infusion of Various Drugs with the Standard Dose of Emulsion A.* (Each values show mean of four samples.)

| Remarks                                                                                | No. of rabbit              | Blood     |               |               |               |               |               |               |               |               |                     | Urine        |  |
|----------------------------------------------------------------------------------------|----------------------------|-----------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------------|--------------|--|
|                                                                                        |                            | 0         | 1hrs.         | 3hrs.         | 6hrs.         | 9hrs.         | 12hrs.        | 18hrs.        | 24hrs.        | 30hrs.        | Concentration mg/dl | Excretion mg |  |
| Emulsion A + Methionine                                                                | Mean (mg/dl)<br>Change (%) | 0.45<br>0 | 1.03<br>+ 129 | 1.56<br>+ 247 | 1.90<br>+ 322 | 2.00<br>+ 344 | 2.22<br>+ 393 | 2.04<br>+ 354 | 1.67<br>+ 271 | 2.29<br>+ 409 | 3.03                | 2.81         |  |
| Emulsion A + Riboflavin                                                                | Mean (mg/dl)<br>Change (%) | 0.76<br>0 | 1.07<br>+ 41  | 1.24<br>+ 63  | 1.19<br>+ 57  | 1.48<br>+ 95  | 0.97<br>+ 28  | 1.11<br>+ 46  | 1.07<br>+ 41  | 1.41<br>+ 86  | 2.28                | 2.17         |  |
| Emulsion A + Ascorbic acid                                                             | Mean (mg/dl)<br>Change (%) | 0.52<br>0 | 1.08<br>+ 108 | 1.47<br>+ 183 | 1.65<br>+ 217 | 1.72<br>+ 231 | 1.94<br>+ 273 | 1.70<br>+ 227 | 2.03<br>+ 290 | 2.32<br>+ 346 | 3.33                | 2.34         |  |
| Emulsion A + Pantothenic acid                                                          | Mean (mg/dl)<br>Change (%) | 0.88<br>0 | 1.48<br>+ 68  | 1.19<br>+ 35  | 1.40<br>+ 59  | 1.47<br>+ 67  | 1.39<br>+ 58  | 1.52<br>+ 73  | 1.59<br>+ 81  | 1.77<br>+ 101 | 2.89                | 2.22         |  |
| Emulsion A + Riboflavin + Niacin amide                                                 | Mean (mg/dl)<br>Change (%) | 0.71<br>0 | 1.15<br>+ 62  | 1.54<br>+ 117 | 1.66<br>+ 134 | 1.80<br>+ 154 | 1.77<br>+ 149 | 1.63<br>+ 130 | 1.54<br>+ 117 | 1.60<br>+ 125 | 2.22                | 1.97         |  |
| Emulsion A + Methionine + Riboflavin                                                   | Mean (mg/dl)<br>Change (%) | 0.73<br>0 | 1.48<br>+ 103 | 1.24<br>+ 70  | 0.98<br>+ 34  | 1.01<br>+ 38  | 0.98<br>+ 34  | 1.32<br>+ 81  | 1.56<br>+ 114 | 1.69<br>+ 132 | 1.93                | 2.21         |  |
| Emulsion A + Methionine + Ascorbic acid                                                | Mean (mg/dl)<br>Change (%) | 0.88<br>0 | 0.97<br>+ 10  | 0.96<br>+ 9   | 1.05<br>+ 19  | 1.47<br>+ 67  | 1.66<br>+ 89  | 1.81<br>+ 106 | 1.76<br>+ 100 | 1.22<br>+ 39  | 2.04                | 1.94         |  |
| Emulsion A + Riboflavin + Ascorbic acid                                                | Mean (mg/dl)<br>Change (%) | 0.59<br>0 | 1.05<br>+ 78  | 1.11<br>+ 88  | 1.25<br>+ 112 | 1.34<br>+ 127 | 1.36<br>+ 131 | 2.03<br>+ 244 | 1.93<br>+ 227 | 1.57<br>+ 166 | 1.87                | 1.87         |  |
| Emulsion A + Methionine + Riboflavin + Ascorbic acid                                   | Mean (mg/dl)<br>Change (%) | 0.76<br>0 | 0.98<br>+ 29  | 1.21<br>+ 59  | 1.39<br>+ 83  | 1.34<br>+ 76  | 0.79<br>+ 4   | 0.90<br>+ 18  | 1.64<br>+ 116 | 2.01<br>+ 164 | 1.70                | 1.52         |  |
| Emulsion A + Methionine + Riboflavin + Niacin amide + Ascorbic acid                    | Mean (mg/dl)<br>Change (%) | 0.64<br>0 | 0.87<br>+ 36  | 1.19<br>+ 86  | 0.99<br>+ 55  | 1.01<br>+ 58  | 1.62<br>+ 153 | 1.53<br>+ 139 | 1.58<br>+ 147 | 1.50<br>+ 134 | 1.77                | 1.58         |  |
| Emulsion A + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid | Mean (mg/dl)<br>Change (%) | 0.88<br>0 | 1.17<br>+ 33  | 1.06<br>+ 20  | 0.94<br>+ 7   | 1.07<br>+ 22  | 1.00<br>+ 14  | 1.17<br>+ 33  | 0.85<br>- 3   | 1.25<br>+ 42  | 1.31                | 1.18         |  |

riboflavin, 2 mg, ascorbic acid, 10 mg, niacin amide, 4 mg and pantothenic acid, 5 mg per kg of body weight were administered intravenously into rabbits in various combinations. The results are presented *en bloc* in Table XI and Fig. 3. The metabolic process of the infused fat emulsion improved merely by the simultaneous infusion of methionine, riboflavin and ascorbic acid with the fat emulsion, in consequence, urinary ketone body excretion decreased markedly. The decrease in blood ketone bodies levels, however, was insufficient. Further addition of niacin amide and pantothenic acid to these three drugs was necessary. Thus blood and urine ketone bodies presented no significant difference from the controls. It is accepted that fatty acids, contained in the infused fat emulsion, enter into the T.C.A. cycle, in which fatty acids are finally oxidized by means of action of these drugs with the most nutritious effect.

(b) Repeated Infusions into Rabbits

Each of the following series of drugs were daily administered intravenously for 20 days into normal rabbits fed by the standard diet under identical conditions as the single infusion experiments; 7 per cent glucose solution, Emulsion A alone, simultaneous combinations of the above mentioned various drugs with Emulsion A. Similar results to those in the single infusion experiments were obtained (Table X, XI, XII and XIII). As shown in Table XIV the rate of body weight gain in the group, which was given simultaneous infusions of methionine, riboflavin and ascorbic acid with the fat emulsion, was higher than that in the group of infusions of the fat emulsion alone. In the group given the simultaneous infusions of methionine, riboflavin, niacin amide, ascorbic acid and pantothenic acid with the fat emulsion the rate of body weight gain was the highest. According to these results, the im-

Table X. Ketone Bodies Levels Following Daily Intravenous Infusions of 7 Per Cent Glucose Solution (3.3cc per kg) for 20 Days.

|       |               | No. of rabbit | Days of infusion |       |       |       |       |       | Mean       |
|-------|---------------|---------------|------------------|-------|-------|-------|-------|-------|------------|
|       |               |               | 0                | 4     | 8     | 12    | 16    | 20    |            |
| Blood |               |               | mg/dl            | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl      |
|       | 100           |               | 0.94             | 0.58  | 1.07  | 0.45  | 1.16  | 1.16  | 0.92±0.068 |
|       | 103           |               | 1.16             | 0.58  | 0.68  | 0.45  | 1.33  | 0.45  |            |
|       | 105           |               | 0.68             | 1.40  | 1.42  | 1.50  | 1.15  | 0.45  |            |
| Urine | Concentration | 102           | 0.45             | 0.45  | 0.91  | 0.58  | 0.83  | 1.06  | 0.89±0.053 |
|       |               | 103           | 0.97             | 0.91  | 0.47  | 0.68  | 1.18  | 1.00  |            |
|       |               | 105           | 0.43             | 0.83  | 0.91  | 1.74  | 0.89  | 0.90  |            |
|       | Excretion     |               | mg               | mg    | mg    | mg    | mg    | mg    | mg         |
|       |               | 102           | 1.40             | 1.40  | 2.28  | 1.62  | 2.24  | 2.65  | 2.22±0.110 |
|       |               | 103           | 2.72             | 2.28  | 2.11  | 1.63  | 3.54  | 2.80  |            |
|       |               | 105           | 0.86             | 1.74  | 1.82  | 3.48  | 2.05  | 1.62  |            |

Table XI. Ketone Bodies Levels Following Daily Intravenous Infusion of the Standard Dose of Emulsion A.

|       |                    | No. of rabbit | Days of infusion |       |       |       |       |       | Mean       |
|-------|--------------------|---------------|------------------|-------|-------|-------|-------|-------|------------|
|       |                    |               | 0                | 4     | 8     | 12    | 16    | 20    |            |
| Blood |                    |               | mg/dl            | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl      |
|       |                    | 101           | 1.30             | 0.69  | 1.07  | 1.45  | 0.82  | 0.68  |            |
|       |                    | 102           | 0.91             | 0.98  | 1.43  | 1.00  | 1.36  | 0.45  | 1.05±0.054 |
|       |                    | 104           | 1.03             | 1.38  | 1.16  | 1.02  | 0.85  | 1.42  |            |
| Urine | Concent-<br>ration | 101           | 0.33             | 1.06  | 3.96  | 1.57  | 1.45  | 1.61  | 1.87±0.133 |
|       |                    | 102           | 0.96             | 1.06  | 3.06  | 1.89  | 1.96  | 1.59  |            |
|       |                    | 104           | 1.13             | 1.80  | 1.30  | 1.79  | 2.07  | 1.84  |            |
|       | Excretion          |               | mg               | mg    | mg    | mg    | mg    | mg    | mg         |
|       |                    | 101           | 2.11             | 4.56  | 11.93 | 5.10  | 5.29  | 4.77  | 5.26±0.444 |
|       |                    | 102           | 0.89             | 4.45  | 11.09 | 5.34  | 3.63  | 3.22  |            |
|       | 104                | 3.05          | 4.50             | 2.73  | 4.12  | 4.14  | 4.05  |       |            |

**Table XII.** *Ketone Bodies Levels Following Daily Simultaneous Infusions of Methionine (5 mg per kg), Riboflavin (2 mg per kg) and Ascorbic Acid (10 mg per kg) with the Standard Dose of Emulsion A for 20 Days.*

|       |                | No. of rabbit | Days of infusion |       |       |       |       |            | Mean       |
|-------|----------------|---------------|------------------|-------|-------|-------|-------|------------|------------|
|       |                |               | 0                | 4     | 8     | 12    | 16    | 24         |            |
| Blood |                |               | mg/dl            | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl      | mg/dl      |
|       | 107            | 0.20          | 1.45             | 1.19  | 0.85  | 1.16  | 0.82  | 1.03±0.066 |            |
|       | 108            | 1.13          | 1.42             | 1.42  | 1.16  | 0.54  | 0.58  |            |            |
|       | 111            | 1.16          | 1.30             | 0.66  | 0.58  | 0.89  | 1.42  |            |            |
| Urine | Concent-ration | 107           | 0.96             | 1.07  | 1.06  | 0.89  | 0.82  | 1.16       | 1.00±0.033 |
|       |                | 108           | 0.56             | 1.06  | 1.07  | 0.89  | 0.91  | 1.15       |            |
|       |                | 111           | 0.43             | 1.17  | 0.66  | 1.16  | 0.62  | 1.29       |            |
|       | Excretion      |               | mg               | mg    | mg    | mg    | mg    | mg         | mg         |
|       |                | 107           | 2.11             | 2.78  | 3.98  | 2.05  | 2.21  | 2.90       | 2.43±0.101 |
|       |                | 108           | 1.68             | 2.65  | 2.68  | 2.40  | 2.28  | 1.84       |            |
|       |                | 111           | 1.16             | 2.34  | 1.52  | 2.67  | 1.61  | 2.58       |            |

**Table XIII.** *Ketone Bodies Levels Following Daily Simultaneous Infusions of Methionine (5 mg per kg), Riboflavin (2 mg per kg), Ascorbic Acid (10 mg per kg), Niacin Amide (4 mg per kg) and Pantothenic Acid (5 mg per kg) with the Standard Dose of Emulsion A for 20 Days.*

|       |               | No. of rabbit | Days of infusion |       |       |       |       |       | Mean       |            |
|-------|---------------|---------------|------------------|-------|-------|-------|-------|-------|------------|------------|
|       |               |               | 0                | 4     | 8     | 12    | 16    | 20    |            |            |
| Blood |               |               | mg/dl            | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl      |            |
|       |               | 109           | 1.16             | 1.40  | 1.13  | 0.20  | 0.68  | 1.40  | 1.12±0.066 |            |
|       |               | 110           | 1.16             | 0.94  | 1.33  | 0.45  | 1.28  | 1.42  |            |            |
|       |               | 112           | 1.30             | 1.03  | 1.24  | 1.43  | 1.50  | 1.30  |            |            |
| Urine | Concentration | 109           | 0.43             | 1.17  | 0.94  | 1.59  | 0.58  | 0.83  | 0.83±0.071 |            |
|       |               | 110           | 0.70             | 0.45  | 0.47  | 0.94  | 0.47  | 1.00  |            |            |
|       |               | 112           | 1.06             | 0.44  | 0.87  | 1.67  | 0.20  | 0.83  |            |            |
|       | Excretion     |               | mg               | mg    | mg    | mg    | mg    | mg    | mg         |            |
|       |               |               | 109              | 1.16  | 3.63  | 2.35  | 3.12  | 1.22  | 1.49       | 1.78±0.151 |
|       |               |               | 110              | 1.40  | 1.26  | 1.03  | 1.88  | 1.03  | 2.00       |            |
|       |               |               | 112              | 2.65  | 0.92  | 1.74  | 3.00  | 0.54  | 1.49       |            |

portance of these vitamins, which were considered to play an essential role in fat metabolism *in vitro*, was established also *in vivo*.

### 3. Examination on the Relationship between Carbohydrate and Fat Metabolism

Recently, according to studies on the T.C.A. cycle, it has been clarified that three major foodstuffs enter into this common metabolic pathway to be oxidized finally. It has been found that acetyl-CoA formed by fatty acid oxidation condenses with oxaloacetic acid to enter into the T.C.A. cycle.<sup>99</sup> Therefore, when the fat emulsion is administered intravenously, we must of course examine its relationship to carbohydrate metabolism, particularly, whether the intravenous administration of the fat emulsion is likewise nutritiously effective or not in a state of disturbed car-

Table XIV. *Changes of Body Weight in the Repeated Infusions.*

| Remarks                                                                                         | No. of rabbit | Initial    | Final      | Increase (mean) |                  |
|-------------------------------------------------------------------------------------------------|---------------|------------|------------|-----------------|------------------|
| Control                                                                                         | 100           | g<br>1,700 | g<br>2,000 | g<br>270        | per cent<br>15.2 |
|                                                                                                 | 103           | 1,700      | 1,950      |                 |                  |
|                                                                                                 | 105           | 1,850      | 2,100      |                 |                  |
| Emulsion A                                                                                      | 101           | 1,750      | 1,950      | 260             | 15.1             |
|                                                                                                 | 102           | 1,750      | 2,150      |                 |                  |
|                                                                                                 | 104           | 1,800      | 2,000      |                 |                  |
| Emulsion A + Methionine +<br>Riboflavin + Ascorbic acid                                         | 107           | 1,750      | 2,050      | 300             | 17.6             |
|                                                                                                 | 108           | 1,750      | 1,950      |                 |                  |
|                                                                                                 | 111           | 1,600      | 2,000      |                 |                  |
| Emulsion A + Methionine +<br>Riboflavin + Niacin amide +<br>Ascorbic acid + Pantothenic<br>acid | 109           | 1,700      | 2,100      | 320             | 18.8             |
|                                                                                                 | 110           | 1,600      | 1,950      |                 |                  |
|                                                                                                 | 112           | 1,750      | 1,950      |                 |                  |

bohydrate metabolism. If not nutritiously effective, it is very important for the further clinical application of the fat emulsion that we correct its defects. In this case Emulsion B was generally used. In case of necessity, the addition of glucose was made at various rate.

(a) *Ketone Body Metabolism in Closed Circuit Anesthesia*

It has already been observed that ether anesthesia causes not only hepatic damage<sup>2, 23)</sup> and accumulation of carbohydrate<sup>6, 7, 21, 48)</sup> or fat intermediates<sup>11, 48, 62, 77, 81)</sup> in blood, but also disturbance of protein metabolism.<sup>39)</sup> Adult dogs each weighting approximately 10 kg were used. After basic narcosis with 2 per cent narcocon, 1 cc and induction of anesthesia with 2.5 per cent pentothal sodium solution, 7 to 10 cc, closed circuit anesthesia with ether was performed at third stage, second plane for four hours. Blood ketone bodies levels, as shown in Table XV, increased remarkably not only during but after anesthesia. KUROKAWA<sup>49)</sup> reported that blood levels of pyruvic acid and lactic acid also increased in these cases. These facts indicate that disturbances of carbohydrate and fat metabolism are caused by ether anesthesia. After thiamin, 1 mg per kg of body weight, was subcutaneously injected into dogs, closed circuit anesthesia was performed as described above. The results are shown in Table XV; blood ketone bodies levels markedly diminished as well as carbohydrate intermediates.<sup>48)</sup> When riboflavin, 1 mg and ascorbic acid, 10 mg per kg of body weight were subcutaneously injected into dogs before anesthesia, blood ketone bodies levels decreased as in the case of thiamin injection (Table XV). On the contrary, carbohydrate intermediates accumulated.<sup>48)</sup> Prior injection of these three vitamins, as shown in Table XV, could prevent the occurrence of not only of carbohydrate but fat metabolism.

It is evident, however, from Table XVI that this result is applicable only in the case of normal dogs but is not applicable in the case of dogs, in which glycogen

**Table XV.** *Blood Ketone Bodies Levels in Anesthetized Normal Dogs.*  
(Various drugs were injected previously.)

| Remarks                                    | No. of dog       | Body weight | Used ether | 0             | 2 hrs.        | 4 hrs.        | 11 hrs.       |
|--------------------------------------------|------------------|-------------|------------|---------------|---------------|---------------|---------------|
| Control                                    | 1                | kg<br>7.5   | cc<br>40   | mg/dl<br>0.56 | mg/dl<br>1.64 | mg/dl<br>2.86 | mg/dl<br>2.35 |
|                                            | 2                | 8.0         | 45         | 0.66          | 1.12          | 2.61          | 2.86          |
|                                            | 3                | 7.5         | 30         | 1.53          | 0.66          | 2.86          | 1.90          |
|                                            | Mean             |             |            | 0.92          | 1.14          | 2.78          | 2.37          |
|                                            | Change, per cent |             |            | 0             | + 24          | + 202         | + 158         |
| Thiamin                                    | 4                | 9.5         | 20         | 0.45          | 1.05          | 1.70          | 1.79          |
|                                            | 5                | 10.5        | 15         | 1.08          | 1.17          | 1.17          | 2.47          |
|                                            | 7                | 9.0         | 20         | 1.12          | 1.77          | 1.35          | 1.33          |
|                                            | Mean             |             |            | 0.88          | 1.33          | 1.41          | 1.86          |
|                                            | Change, per cent |             |            | 0             | + 51          | + 60          | + 111         |
| Riboflavin +<br>Ascorbic acid              | 9                | 9.5         | 30         | 1.04          | 1.51          | 1.51          | 4.61          |
|                                            | 10               | 8.5         | 15         | 0.45          | 1.10          | 1.10          | 3.13          |
|                                            | 12               | 10.0        | 20         | 1.18          | 1.54          | 1.28          | 1.40          |
|                                            | Mean             |             |            | 0.89          | 1.38          | 1.30          | 3.05          |
|                                            | Change, per cent |             |            | 0             | + 55          | + 46          | + 243         |
| Thiamin +<br>Riboflavin +<br>Ascorbic acid | 13               | 9.8         | 40         | 1.26          | 1.14          | 0.66          | 1.14          |
|                                            | 14               | 10.0        | 25         | 0.87          | 1.27          | 1.14          | 0.87          |
|                                            | 15               | 10.5        | 30         | 0.46          | 1.14          | 1.21          | 1.19          |
|                                            | Mean             |             |            | 0.86          | 1.10          | 1.00          | 1.07          |
|                                            | Change, per cent |             |            | 0             | + 28          | + 16          | + 24          |

**Table XVI.** *Blood Ketone Bodies Levels in Anesthetized Starved Dogs.*  
(Various drugs were injected previously.)

| Remarks                                                           | No. of dog       | Body weight | Used ether | 0     | 2 hrs. | 4 hrs. | 11 hrs. |
|-------------------------------------------------------------------|------------------|-------------|------------|-------|--------|--------|---------|
| Thiamin +<br>Riboflavin +<br>Ascorbic acid                        |                  | kg          | cc         | mg/dl | mg/dl  | mg/dl  | mg/dl   |
|                                                                   | 16               | 8.5         | 25         | 1.39  | 2.58   | 2.81   | 1.39    |
|                                                                   | 17               | 10.0        | 26         | 0.89  | 2.42   | 2.40   | 1.68    |
|                                                                   | 18               | 9.3         | 25         | 0.21  | 1.66   | 3.43   | 5.30    |
|                                                                   | Mean             |             |            | 0.83  | 2.22   | 2.88   | 2.79    |
| Change, per cent                                                  |                  |             | 0          | + 167 | + 247  | + 236  |         |
| Thiamin + Riboflavin<br>+ Ascorbic acid +<br>20% glucose solution | 19               | 9.8         | 30         | 1.57  | 2.21   | 0.83   | 1.30    |
|                                                                   | 20               | 10.0        | 30         | 0.76  | 0.92   | 0.76   | 1.15    |
|                                                                   | Mean             |             |            | 1.17  | 1.57   | 0.80   | 1.23    |
|                                                                   | Change, per cent |             |            | 0     | + 34   | - 32   | + 5     |

depots appeared to be exhausted after fasting for 5 days. When subcutaneous injection of these three vitamins and 20 per cent glucose solution was made 30 minutes before anesthesia, as shown in Table XVI, blood ketone bodies levels did not increase without accumulation of carbohydrate intermediates<sup>49)</sup> even in the case of starved anesthetized dogs. These results indicate that disturbance of fat metabolism in closed circuit anesthesia is a secondary change due to defective carbohydrate metabolism. Therefore the injection of thiamin into normal dogs or that of both thiamin and glucose into starved dogs, in which glycogen depots were diminished, was made. Carbohydrate metabolism was improved and the formation of oxaloacetic acid, which was necessary for entrance of acetyl-CoA into the T.C.A. cycle, was accelerated. This then represents a radical method for the improvement of disturbances of both carbohydrate and fat metabolism in closed circuit anesthesia. If possible, simultaneous injection of riboflavin and ascorbic acid is preferable. These results demonstrate *in vivo* that carbohydrate and fat metabolism are closely related to each other.

(b) *Is Simultaneous Infusion of Glucose Necessary or not for the Intravenous Administration of the Fat Emulsion into Normal and Starved Rabbits?*

*In Normal Rabbits* — Viewed from the point of the decrease of blood ketone bodies levels and their urinary excretion, the simultaneous infusion of the above mentioned drugs with Emulsion A was more than that with Emulsion B, while in the case of the infusion of the fat emulsion alone there was no significant difference between these two fat emulsions (Table XVII). Accordingly, application of the emulsion containing 7 per cent glucose (Emulsion A) is necessary for the in-

**Table XVII.** *Ketone Bodies Levels Following Intravenous Infusion of Emulsion B (3.3 cc per kg) and Various Drugs into Normal Rabbits.*

| Remarks                                                                                | No. of rabbit | Blood         |               |               |               |               |               |               |               |               |               | Urine      |  |
|----------------------------------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|------------|--|
|                                                                                        |               | 0             | 1hrs.         | 3hrs.         | 6hrs.         | 9hrs.         | 12hrs.        | 18hrs.        | 24hrs.        | 30hrs.        | Concentration | Excretion  |  |
| Emulsion B                                                                             | 128           | mg/dl<br>0.45 | mg/dl<br>1.18 | mg/dl<br>1.61 | mg/dl<br>2.27 | mg/dl<br>1.94 | mg/dl<br>2.51 | mg/dl<br>1.94 | mg/dl<br>2.12 | mg/dl<br>1.61 | mg/dl<br>3.00 | mg<br>3.00 |  |
|                                                                                        | 129           | 0.57          | 0.85          | 0.93          | 1.67          | 1.69          | 2.12          | 1.95          | 1.71          | 1.72          | 3.03          | 2.73       |  |
|                                                                                        | 157           | 0.67          | 1.18          | 1.68          | 1.45          | 1.72          | 2.45          | 1.95          | 2.66          | 2.15          | 5.21          | 4.17       |  |
|                                                                                        | 158           | 0.54          | 1.01          | 1.73          | 1.73          | 1.98          | 2.64          | 1.61          | 1.50          | 1.74          | 2.86          | 2.86       |  |
|                                                                                        | Mean          | 0.56          | 1.06          | 1.49          | 1.78          | 1.83          | 2.43          | 1.86          | 2.00          | 1.81          | 3.53          | 3.19       |  |
|                                                                                        | Change(%)     | 0             | +89           | +116          | +218          | +226          | +334          | +232          | +257          | +223          |               |            |  |
| Emulsion B + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid | 130           | 0.92          | 0.95          | 1.44          | 1.85          | 1.71          | 1.67          | 1.45          | 1.19          | 1.34          | 1.95          | 1.95       |  |
|                                                                                        | 131           | 0.86          | 1.11          | 1.84          | 1.84          | 1.73          | 2.04          | 2.09          | 1.89          | 1.40          | 2.58          | 2.05       |  |
|                                                                                        | 133           | 0.69          | 1.14          | 1.45          | 1.72          | 1.74          | 2.07          | 2.29          | 1.89          | 1.68          | 1.79          | 1.97       |  |
|                                                                                        | 134           | 0.46          | 0.95          | 1.45          | 1.72          | 1.14          | 1.45          | 1.72          | 1.44          | 1.44          | 4.57          | 2.28       |  |
|                                                                                        | Mean          | 0.73          | 1.04          | 1.55          | 1.78          | 1.58          | 1.81          | 1.89          | 1.60          | 1.47          | 2.72          | 2.06       |  |
|                                                                                        | Change(%)     | 0             | +43           | +112          | +144          | +116          | +148          | +159          | +119          | +101          |               |            |  |

travenous administration into normal rabbits.

*In Starved Rabbits* — In total starvation glycogen depots are first exhausted and animals derive energy from both fat and protein. In this state the liver oxidizes successfully fatty acids mobilized from fat depots.<sup>74)</sup> Moreover, carbohydrates, especially oxaloacetic acid, which is necessary for direct or indirect entrance of large amounts of formed acetyl-CoA into the T.C.A. cycle, has been already lacking. Accordingly blood ketone bodies levels and their urinary excretion increase. When fat depots are exhausted, the formation of ketone bodies decreases and destruction of tissue protein occurs rapidly and intensely.<sup>74)</sup> Of course, patients with malnutrition are the object of our clinical application of parenteral nutrition with the fat emulsion. In these cases we can not determine the dose of the fat emulsion on the base of the results only in normal cases. This problem was ascertained experimentally.

Since the hepatic glycogen content reaches the minimal level in 5.5 th day of starvation,<sup>35,39)</sup> fasted rabbits for 5 days were used in the present starvation experiment. RINGER's solution, 3.3 c.c. per kg of body weight, was administered intravenously into starved rabbits as the controls. It was observed that one group showed high blood ketone bodies levels and another showed relatively low (Table XVIII). It was thought that the former group had preserved fat depots, while the latter almost had more nearly exhausted them. Each of the following combinations were intravenously administered into starved rabbits. Emulsion A alone, Emulsion B alone, and the simultaneous combinations of above mentioned various drugs with each of these two fat emulsions. Marked decrease in ketone bodies levels was not observed (Table XVIII, XIX). Not only methionine, 5 mg, riboflavin, 2 mg, niacin amide, 4 mg,

**Table XVIII.** *Effect of Addition of Glucose to the Fat Emulsion on Blood Ketone Bodies Levels.*  
(Ringer's solution or the fat emulsion was intravenously administered into starved rabbits.)

| Remarks           | No.<br>of<br>rabbit | Time after infusion (hrs.) |               |               |               |               |               |               |               |
|-------------------|---------------------|----------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                   |                     | 0                          | 1             | 3             | 6             | 9             | 12            | 18            | 24            |
| Ringer's solution | 135                 | mg/dl<br>1.60              | mg/dl<br>1.66 | mg/dl<br>1.63 | mg/dl<br>2.09 | mg/dl<br>1.96 | mg/dl<br>1.03 | mg/dl<br>1.68 | mg/dl<br>1.93 |
|                   | 136                 | 1.72                       | 1.43          | 1.76          | 1.71          | 1.72          | 1.52          | 1.14          | 0.95          |
|                   | 141                 | 0.55                       | 1.07          | 1.19          | 1.14          | 1.08          | 1.42          | 1.18          | 0.99          |
|                   | Mean                | 1.29                       | 1.39          | 1.53          | 1.65          | 1.59          | 1.32          | 1.33          | 1.29          |
| Emulsion B        | 137                 | 1.61                       | 1.39          | 1.19          | 1.63          | 2.41          | 2.14          | 2.45          | 2.93          |
|                   | 138                 | 2.11                       | 1.16          | 1.32          | 1.84          | 2.09          | 2.95          | 2.69          | 2.35          |
|                   | 139                 | 2.45                       | 1.77          | 1.71          | 1.78          | 2.38          | 2.62          | 2.05          | 2.22          |
|                   | Mean                | 2.06                       | 1.44          | 1.41          | 1.75          | 2.29          | 2.57          | 2.40          | 2.50          |
| Emulsion A        | 140                 | 2.60                       | 2.00          | 2.62          | 1.96          | 2.17          | 3.04          | 2.73          | 3.12          |
|                   | 143                 | 1.65                       | 1.32          | 1.19          | 1.42          | 1.45          | 1.82          | 1.86          | 2.08          |
|                   | 144                 | 0.92                       | 1.27          | 0.89          | 2.49          | 2.90          | 1.68          | 1.72          | 1.42          |
|                   | Mean                | 1.72                       | 1.53          | 1.57          | 1.96          | 2.17          | 2.18          | 2.10          | 2.21          |

**Table XX.** *Effect of Addition of Glucose and Various Drugs to the Fat Emulsion on Blood Ketone Bodies Levels.*

(Various drugs were intravenously administered simultaneously with the standard dose of Emulsion A or Emulsion B into starved rabbits.)

| Remarks                                                                                                      | No. of rabbit | Time after infusion (hrs.) |            |            |            |            |            |            |            |
|--------------------------------------------------------------------------------------------------------------|---------------|----------------------------|------------|------------|------------|------------|------------|------------|------------|
|                                                                                                              |               | 0                          | 1          | 3          | 6          | 9          | 12         | 18         | 24         |
| EmulsionB + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid                        | 145           | mg/dl 1.63                 | mg/dl 1.45 | mg/dl 1.45 | mg/dl 1.71 | mg/dl 2.06 | mg/dl 1.35 | mg/dl 1.68 | mg/dl 1.85 |
|                                                                                                              | 146           | 1.80                       | 1.78       | 1.40       | 1.73       | 1.63       | 1.05       | 2.42       | 1.53       |
|                                                                                                              | 152           | 1.45                       | 1.38       | 1.75       | 1.68       | 1.42       | 1.42       | 1.72       | 2.42       |
|                                                                                                              | Mean          | 1.63                       | 1.54       | 1.53       | 1.71       | 1.70       | 1.27       | 1.94       | 1.93       |
|                                                                                                              |               |                            |            |            |            |            |            |            |            |
| EmulsionA + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid                        | 153           | 1.60                       | 1.45       | 1.08       | 2.08       | 1.05       | 2.09       | 1.68       | 1.62       |
|                                                                                                              | 155           | 1.45                       | 1.12       | 1.35       | 1.78       | 2.93       | 1.90       | 1.68       | 1.05       |
|                                                                                                              | 156           | 1.82                       | 1.18       | 1.59       | 1.18       | 1.60       | 2.11       | 1.68       | 2.18       |
|                                                                                                              | Mean          | 1.62                       | 1.25       | 1.34       | 1.68       | 1.86       | 2.03       | 1.68       | 1.62       |
|                                                                                                              |               |                            |            |            |            |            |            |            |            |
| EmulsionA + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid + 20% glucose solution | 160           | 0.92                       | 0.44       | 1.11       | 1.43       | 1.71       | 1.71       | 1.17       | 1.35       |
|                                                                                                              | 162           | 1.68                       | 0.83       | 1.05       | 1.19       | 1.08       | 1.86       | 1.40       | 1.73       |
|                                                                                                              | 165           | 1.72                       | 0.68       | 0.46       | 1.63       | 1.18       | 1.68       | 1.14       | 1.43       |
|                                                                                                              | Mean          | 1.44                       | 0.65       | 0.87       | 1.42       | 1.32       | 1.75       | 1.24       | 1.50       |
|                                                                                                              |               |                            |            |            |            |            |            |            |            |

**Table XX.** *Blood Sugar Levels in Starved Rabbits.*  
(All values are in milligram per cent.)

| Remarks                                                                                                      | No. of rabbit | 0   | 1 hrs. | 3 hrs. | 6 hrs. | 9 hrs. | 12 hrs. | 18 hrs. | 24 hrs. |
|--------------------------------------------------------------------------------------------------------------|---------------|-----|--------|--------|--------|--------|---------|---------|---------|
| Ringer's solution                                                                                            | 135           | 60  | 60     | 53     | 60     | 55     | 60      | 50      | 55      |
|                                                                                                              | 136           | 58  | 98     | 56     | 58     | 68     | 55      | 54      | 56      |
|                                                                                                              | 141           | 60  | 70     | 65     | 70     | 58     | 60      | 60      | 50      |
| Emulsion B                                                                                                   | 137           | 49  | 59     | 167    | 52     | 77     | 50      | 60      | 72      |
|                                                                                                              | 138           | 105 | 94     | 56     | 49     | 50     | 48      | 50      | 61      |
|                                                                                                              | 139           | 70  | 72     | 65     | 70     | 65     | 45      | 60      | 50      |
| Emulsion A                                                                                                   | 140           | 70  | 72     | 90     | 70     | 70     | 85      | 45      | 70      |
|                                                                                                              | 143           | 99  | 69     | 80     | 113    | 90     | 90      | 50      | 128     |
|                                                                                                              | 144           | 88  | 105    | 105    | 82     | 88     | 116     | 60      | 80      |
| EmulsionB + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid                        | 145           | 46  | 46     | 50     | 82     | 92     | 53      | 60      | 60      |
|                                                                                                              | 146           | 78  | 95     | 78     | 72     | 54     | 68      | 65      | 55      |
|                                                                                                              | 152           | 54  | 57     | 68     | 48     | 50     | 50      | 52      | 50      |
| EmulsionA + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid                        | 153           | 76  | 114    | 80     | 57     | 54     | 68      | 70      | 57      |
|                                                                                                              | 155           | 57  | 90     | 50     | 50     | 48     | 50      | 60      | 46      |
|                                                                                                              | 156           | 63  | 80     | 50     | 53     | 53     | 57      | 60      | 63      |
| EmulsionA + Methionine + Riboflavin + Niacin amide + Ascorbic acid + Pantothenic acid + 20% glucose solution | 160           | 63  | 206    | 74     | 63     | 46     | 52      | 60      | 63      |
|                                                                                                              | 162           | 70  | 175    | 80     | 80     | 57     | 60      | 52      | 55      |
|                                                                                                              | 165           | 60  | 170    | 80     | 80     | 87     |         |         |         |

ascorbic acid, 10 mg and pantothenic acid, 5 mg but also 20 per cent glucose solution, 3.3 cc and thiamin, 2 mg per kg of body weight were intravenously administered simultaneously with the standard dose of Emulsion A. Blood ketone bodies levels increased slightly after a remarkable decrease, but there was no difference from the controls. These results are summarized in Table XIX. In Table XX the blood sugar levels in the present starvation experiment are recorded.

When the standard dose of 15 per cent sesame oil emulsion is intravenously administered into normal rabbits, the addition of 7 per cent glucose is sufficient, and in starved rabbits the addition of larger quantities of both glucose and thiamin is necessary. Obvious results as in the case of closed circuit anesthesia were not obtained in the present starvation experiment. This is possibly because of the uncertainty of the experimental conditions, and also suggests that oxaloacetic acid, which is necessary for entrance of acetyl-CoA into the T.C.A. cycle, are also formed from some amino acids. Since protein metabolism is disturbed to some degree in ether anesthesia,<sup>39)</sup> it is surmised that in ether anesthesia deficiency of oxaloacetic acid is present to a higher degree than in starvation.

## DISCUSSION

While all short chain fatty acids convert to ketone bodies in the liver,<sup>46,74)</sup> long chain fatty acids are metabolized through the following three pathways.

- (1) transported to fat depots<sup>80,81)</sup>
- (2) oxidized by the extrahepatic tissues<sup>28,55)</sup>

(3) after conversion to acetyl-CoA in the liver, a part of acetyl-CoA is immediately oxidized in the liver and pairs of major part of acetyl-CoA react together to form acetoacetic acid, which diffuses into the blood stream. Both acetyl-CoA and ketone bodies are carried to the extrahepatic tissues, where they are finally oxidized.<sup>46,162)</sup> Accordingly the process of fatty acid oxidation divides into direct oxidation, in which fatty acids are directly oxidized in the tissues,<sup>28,55)</sup> and indirect oxidation, in which they are oxidized finally in the extrahepatic tissues after conversion to ketone bodies.<sup>10,19,43,88,89)</sup> CHAIKOFF and his co-workers<sup>29)</sup> found that after intravenous injection of C<sup>14</sup>-labeled tripalmitin emulsion into hepatectomized dogs, the conversion of C<sup>14</sup>O<sub>2</sub> was less than 40 per cent of that in normal dogs. This fact illustrates that less than 40 per cent of the infused radioactive palmitic acid is oxidized directly by the extrahepatic tissues and more than 60 per cent of that is first disposed in the liver.

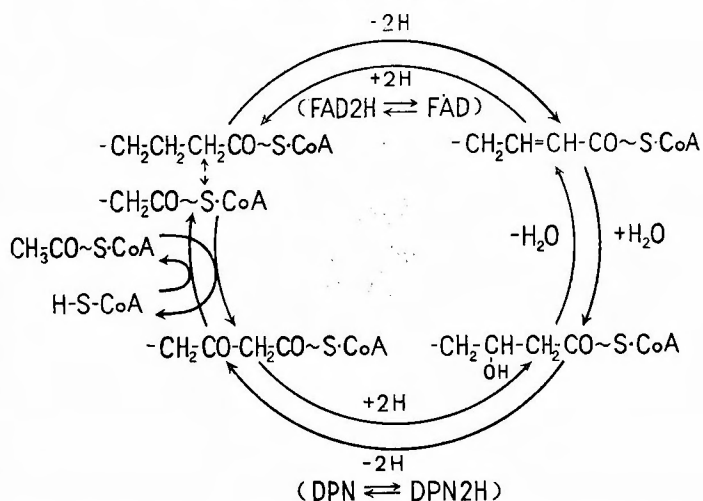
The liver has been considered as the chief site for the formation of ketone bodies according to various experiments applied liver slices,<sup>13,17,17,41,42,43,56,75,90,100)</sup> living animals<sup>30)</sup> and perfusion techniques of the liver.<sup>20,93)</sup> All these experiments, however, were performed using free fatty acids, especially their esters of short chain fatty acids. In the present perfusion experiment with sesame oil emulsion containing triglycerides of long chain fatty acids, it was also found that ketone bodies were formed chiefly in the liver. On the basis of the results of the perfusion experiment and intravenous administration of the fat emulsion and those of previous reports from our labo-

ratory, the metabolic pathway of the fat emulsion has been clarified. Considerable lipemia is observed temporarily following intravenous administration of the fat emulsion. As the infused fat globules are phagocytized by the reticuloendothelial cells, the blood fat content decreases gradually.<sup>36)</sup> As lipoidization of neutral fat progresses in these cells, increase in the hepatic phospholipid content is observed biochemically<sup>37)</sup>. This fact, similar to histochemical findings,<sup>5,40)</sup> illustrated clearly that the major part of the infused fat, which is disposed of by the reticuloendothelial cells, enters into the hepatic parenchymatous cells in the form of phospholipid. Thus fatty acids, which are contained in the fat emulsion, are oxidized in the hepatic parenchymatous cells. Consequently oxygen consumption in the liver increases remarkably.<sup>70)</sup> It is accepted that the part of the fatty acids are oxidized finally to water and carbon dioxide in the liver, but the major part of them are converted to the stage of ketone bodies which diffuse into the blood stream with an increase in ketone bodies levels. When ketone bodies are carried to the extrahepatic tissues to enter into the final metabolic pathway, an increase in oxygen consumption in these tissues is observed.<sup>70)</sup> These results agree with CHAIKOFF et al.<sup>29,55)</sup> and GEYER et al.<sup>27,28)</sup>

Since various enzyme systems are concerned in this metabolic process, we must consider the enzyme systems in order to utilize completely the infused fat emulsion.

Methionine synthesizes choline, which has remarkable lipotropic action,<sup>85,87)</sup> by means of transmethylation. Recently ARTOM<sup>3,4)</sup> and ENTENMAN<sup>100)</sup> have emphasized that choline accelerates fatty acid oxidation in the liver. It has been clarified by the results of the present perfusion experiment and of SENO<sup>82)</sup> that methionine accelerates markedly oxidation of neutral fat at least to the stage of ketone bodies in the liver. Recently, according to biochemical studies *in vitro* on fatty acid oxidation by GREEN, LIPMANN, OCHOA and LYNEN etc., it has been recognized that both pyridine nucleotides and flavoproteins play an essential role as hydrogen carrier in this metabolic process (Fig. 1 and 4). Furthermore, it has been established from their *in vitro* studies that all hydrogen atoms, which are liberated by turn of the T.C.A. cycle, are carried by pyridine nucleotides, flavoproteins and cytochrome systems to combine with oxygen, and thus fatty acid is oxidized completely to water and carbon dioxide.<sup>1,8,50,59)</sup> Since pyridine nucleotide and flavoprotein each contain nicotinic

Fig. 4. Fatty acid cycle (After Lynen<sup>59, 60)</sup>)



the liver. Recently, according to biochemical studies *in vitro* on fatty acid oxidation by GREEN, LIPMANN, OCHOA and LYNEN etc., it has been recognized that both pyridine nucleotides and flavoproteins play an essential role as hydrogen carrier in this metabolic process (Fig. 1 and 4). Furthermore, it has been established from their *in vitro* studies that all hydrogen atoms, which are liberated by turn of the T.C.A. cycle, are carried by pyridine nucleotides, flavoproteins and cytochrome systems to combine with oxygen, and thus fatty acid is oxidized completely to water and carbon dioxide.<sup>1,8,50,59)</sup> Since pyridine nucleotide and flavoprotein each contain nicotinic

acid<sup>(22,24,98)</sup> and flavin,<sup>(105)</sup> both nicotinic acid and flavin are essential components of hydrogen carrying system in fatty acid oxidation. More recently flavin has been isolated from some fatty acid oxidizing enzymes.<sup>(64,83)</sup> We must of course consider the importance of these enzyme system in the case of parenteral nutrition with fat emulsion, while many investigators have regarded only the caloric intake and have left this problem out of consideration. Prolonged injection of sodium acetoacetate into rabbits causes a decrease in not only riboflavin<sup>(69)</sup> but also ascorbic acid in serum<sup>(68)</sup>. It has also been demonstrated that ascorbic acid activates both aconitase<sup>(34)</sup> and succinic dehydrogenase<sup>(71)</sup> and is able to convert acetoacetic acid probably to acetic acid and glycolic acid.<sup>(86)</sup> Therefore, by means of the simultaneous infusion of methionine, riboflavin, niacin amide, ascorbic acid and pantothenic acid with the fat emulsion, the infused fat was oxidized smoothly and completely. Under these conditions the fat emulsion was nutritiously effective and resulted in an increase in body weight even in normal rabbits, in which the utilization of the infused fat was difficult. These facts demonstrate also the importance of these vitamins in catalyzing fat metabolism *in vivo*, which has already been established *in vitro*.

It has been disputed that carbohydrate is ketolytic or anti-*ketogenic*. According to DEUEL's experiment<sup>(12)</sup> applied liver slices and WEINHOUS's findings<sup>(101)</sup> that carbohydrate increases the rate of oxidation of short chain fatty acids. It has been established recently that carbohydrate does not inhibit ketone body formation by competition but has a ketolytic action. Acetyl-CoA, which is formed by  $\beta$ -oxidation of fatty acids, and to which ketone bodies are reconverted, combines with oxaloacetic acid to form citric acid under catalyzation of the condensing enzyme and enters into the T.C.A. cycle and is oxidized to water and carbon dioxide. Oxaloacetic acid is formed by means of WOOD-WERKMAN's reaction<sup>(103)</sup> chiefly from pyruvic acid, which is derived from carbohydrate, and partially from some amino acids. When glycogen depots are exhausted in starvation, the minimal necessary dose of oxaloacetic acid could be supplied merely by some amino acids. Consequently, fat depots are mobilized and fatty acids are oxidized excessively in the liver. Thus excessive formation of acetyl-CoA and depletion of oxaloacetic acid occur simultaneously, and remarkable ketosis appears. As shown in these results of the present investigation, when the fat emulsion is administered clinically into patients with malnutrition, the addition of 7 per cent glucose is insufficient and the addition of larger quantities of glucose and above mentioned various drugs are necessary. Thus the results in closed circuit anesthesia also clarified the relationship between carbohydrate and fat metabolism.

## SUMMARY

1. Perfusion of isolated liver was made by application of sesame oil emulsion containing triglycerides of long chain fatty acids, and it was demonstrated that fatty acids contained in the fat emulsion were oxidized at least to the stage of ketone body in the liver. Methionine accelerated and enhanced this oxidation process of fat metabolism.
2. Cod liver oil emulsion was more *ketogenic* than sesame oil emulsion.

3. It is preferable that the fat emulsion is administered simultaneously with methionine, riboflavin, niacin amide, ascorbic acid and pantothenic acid. The importance of these drugs in fat metabolism was clearly demonstrated.

4. In the case of intravenous administration of the fat emulsion into normal rabbits, the fat emulsion must be added to 7 per cent glucose.

5. In the case of intravenous administration of the fat emulsion into starved rabbits, a larger quantities of glucose and the above mentioned drugs must be infused simultaneously with the fat emulsion. Accordingly these facts must be carefully considered especially in the case of clinical application of the fat emulsion into patients with malnutrition.

6. Fat metabolism from the view-point of ketone body formation was discussed by application of the fat emulsion.

7. Precautions for disturbances of fat metabolism in closed circuit anesthesia were discussed.

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## 和文抄録

# ケトン体を中心とした脂質代謝に関する実験的研究

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教室創製の脂肪乳剤を試験(家兎, 猫, 犬)の静脈内へ注入し,あるいは又本脂肪乳剤による剔出肝臓灌流実験を行い,生体内に於ける脂質代謝過程をケトン体の産生状態から観察し,次の所見を認識することが出来た。

(1) 長鎖脂肪酸のトリグリセライドからなる胡麻油乳剤を以ての剔出肝臓灌流実験の結果,長鎖脂肪酸も亦肝臓に於てケトン体の段階迄酸化され得て,而もメチオンはこの酸化過程を著しく促進せしめる。

(2) 併し生体静脈内注入時の所見からすれば長鎖脂肪酸が facultative ketogenic であるのに反して,短鎖脂肪酸は obligatory ketogenic である。而して(1)の所見と併せ考えると,長鎖脂肪酸は肝臓のみならず他の臓器に於ても酸化され得る事実を推察出来る。

(3) 脂質代謝が円滑に行われるためにはリポフラビ

ン, ビタミンC, ニコチン酸, パントテン酸等の脂肪酸酸化酵素系の存在が必要である事実を *in vivo* に於てもよく端的に立証し得た。従つて本脂肪乳剤の注入に当つては,此等ビタミン類の併用が考慮されるべきである。

(4) 健常試験の静脈内へ脂質を乳化態の型で注入し,その栄養学的効果を期待するためには常に7%程度の糖添加が必要である。

(5) 飢餓試験を対象とした際には7%程度の糖添加では不充分で,更に多量の糖添加を必要とし,更に可能なればビタミンB<sub>1</sub>の併用をも行うことが有利である。

(6) 本脂肪乳剤の静脈内注入,剔出肝臓灌流実験,更に又エーテル使用による全身麻醉法等を駆使して,ケトン体を中心とした脂質代謝機構を生体に於てある程度迄究明し得た。