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### Effect of Sodium Taurocholate on Fat Malabsorption Induced by Feeding Unheated Soybean Proteins.\* (30035)

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Chicks fed diets containing unheated hexane-extracted soybeans absorb fat poorly(1, 2). The portion of the soybean responsible for causing the poor absorption of fat is present in a group of proteins, soluble at pH 4.4 and readily extractable from the soybean, sometimes termed "whey" proteins(3). The causative agent in the soybean is heat labile and only affects chicks under 2 to 3 weeks of age. Chicks 4 weeks old or older normally show little impairment of fat absorption when fed unheated soybean meal preparations(2).

The experiments reported here show that the effect of these soybean proteins on fat absorption in young chicks can be counteracted by including the bile salt, sodium taurocholate, or lecithin in the diet fed to chicks.

**Experimental.** Male Rhode Island Red X Barred Plymouth Rock crossbred chicks from a commercial hatchery were used in all experiments. The chicks were housed in thermostatically controlled battery brooders with raised wire floors and feed and water were supplied *ad libitum*. The absorption of dietary fat was determined by the procedures described by Renner and Hill(4). Excreta collections were made on the last 4 consecutive days of the 2-week experimental period. The diet used was the same described by Nesheim

*et al*(2) and contained soybean meal, glucose, soybean oil, DL-methionine, glycine, vitamin and mineral supplements. Unheated soybean meal was included in the experimental diet at the expense of an equal weight of heated soybean meal. The unheated soybean meal consisted of dehulled, hexane-extracted flaked soybeans which had been subjected to a minimum of heat treatment. The heated soybean meal was commercially prepared 50% protein soybean meal. Brambila *et al*(1) conclusively showed that the factor in soybeans responsible for inducing poor fat absorption in chicks was heat labile.

Total bile acids in bile were determined according to the method of Levin and co-workers(5).

**Results.** The first experiment was conducted to determine whether addition of sodium taurocholate or lecithin to a diet containing unheated soybean meal would affect the absorption of fat by chicks fed this diet. The results are shown in Table I.

Chicks fed the diet containing heated soybean meal absorbed 94% of the ingested fat. Those fed the diet containing unheated soybean meal absorbed only 38% of the ingested fat. Chicks receiving the diet containing unheated soybean meal and 0.6% sodium taurocholate absorbed fat to the same extent as the control chicks receiving the diet containing heated soybean meal. The chicks receiving the diet containing unheated soy-

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TABLE I. Effect of Sodium Taurocholate or Lecithin on the Fat Absorption Defect Caused by Feeding Unheated Soybean Meal.

| Dietary treatment*         | Body wt<br>(2 wk)<br>(g) | Fat absorption<br>(%) |
|----------------------------|--------------------------|-----------------------|
| Heated soybean meal        | 174 <sup>a†</sup>        | 94 <sup>a†</sup>      |
| Unheated " " 20%           | 130 <sup>b</sup>         | 38 <sup>c</sup>       |
| + 0.6% sodium taurocholate | 127 <sup>b</sup>         | 92 <sup>a</sup>       |
| + 3% soybean lecithin      | 138 <sup>b</sup>         | 68 <sup>b</sup>       |

\* Average of 3 replicates of 11 chicks.

† Values not followed by same letter are significantly different ( $P < 0.01$ ) according to Duncan's multiple range test(11).

bean meal plus 3% soybean lecithin also absorbed fat better than chicks receiving the diet containing unheated meal alone. The effectiveness of dietary bile salt and lecithin suggested that chicks receiving unheated soybean meal may not secrete sufficient bile to cause adequate solubilization of the dietary fat.

To confirm the observation made in the experiment shown in Table I, the experiment was repeated and a lower level of sodium taurocholate was studied. In addition, the effects of supplementary dietary cystine or taurine were investigated. Bile acids in chick bile are primarily conjugated with taurine(6). Although the diet used in these studies already contained 0.3% supplementary methionine, the effects of these sulfur compounds were studied because of numerous reports linking impaired sulfur amino acid metabolism and unheated soybean meal feeding (7,8). The results of this experiment are shown in Table II.

Chicks fed the diet containing heated soybean meal absorbed 86% of the ingested fat,

TABLE II. Effect of Sodium Taurocholate, Cystine, and Taurine on Fat Absorption.

| Dietary treatment*         | Body wt<br>(2 wk)<br>(g) | Fat absorption<br>(%) |
|----------------------------|--------------------------|-----------------------|
| Heated soybean meal        | 168 <sup>a†</sup>        | 86 <sup>a†</sup>      |
| Unheated " " 30%           | 125 <sup>b</sup>         | 48 <sup>b</sup>       |
| + .4 % L-cystine           | 131 <sup>b</sup>         | 43 <sup>b</sup>       |
| + .42% taurine             | 125 <sup>b</sup>         | 40 <sup>b</sup>       |
| + .3 % sodium taurocholate | 129 <sup>b</sup>         | 80 <sup>a</sup>       |
| + .6 % " "                 | 123 <sup>b</sup>         | 86 <sup>a</sup>       |

\* Average of 3 replicates of 10 chicks.

† Values not followed by same letters are significantly different ( $P < 0.01$ ) according to Duncan's multiple range test(11).

whereas those fed the diet containing unheated soybean meal absorbed only 48% of the ingested fat. The addition of neither cystine nor taurine to the diet containing unheated soybean meal improved the absorption of fat of chicks fed these diets. Chicks receiving the diets containing unheated soybean meal and 0.3 or 0.6% sodium taurocholate absorbed fat normally.

Although dietary sodium taurocholate supplementation improved absorption of fat in chicks fed unheated soybean meal, the growth rate of these chicks was not affected. This is probably because interference with fat absorption is only one effect observed when chicks are fed diets containing raw soybean meal. The other effects, such as pancreatic hypertrophy and poor protein digestion, were not influenced by feeding the bile salt.

In view of the results obtained with bile salt supplementation of the diet, an additional experiment was conducted to determine if bile secretion or composition appeared to be abnormal in chicks receiving unheated soybean protein. Bile was collected from the gall bladder with a hypodermic syringe after either a 12-hour fast or 5 hours after being fed. Previous to this, chicks were fed diets containing either heated or unheated soybean meal from hatching to 14 days of age. The results obtained are shown in Table III.

The chicks fed the diet containing unheated soybean meal had less total bile than chicks fed the diet containing heated meal regardless of the length of fast. When expressed on a per unit of body weight basis, there was no difference in total volume of bile between the soybean meal treatments. The concentration of bile acids in the bile was the same in both treatments and the difference in total amount of bile acids per chick was again a reflection of the difference in body size. The differences in ability of the chicks to absorb fat when fed the experimental diets were previously shown to be unrelated to differences in growth rate or body size(9).

In the bile taken after feeding there was no significant decrease in total volume of

TABLE III. Analysis of Bile from Chicks Fed Heated or Unheated Soybean Meal.

| Absorptive state | Diet                   | Body wt | Vol of bile | Bile/body wt | Bile acids*  | Total bile acids |
|------------------|------------------------|---------|-------------|--------------|--------------|------------------|
|                  |                        | (g)     | (ml/chick)  | (ml/100 g)   | (mg/ml)      | (mg/chick)       |
| Fasted           | Soybean meal, heated   | 176     | .27         | .15          | 99 $\pm$ 7†  | 27.1 $\pm$ 2.2†  |
|                  | Soybean meal, unheated | 127     | .20         | .16          | 100 $\pm$ 5  | 19.5 $\pm$ 1.2   |
| Fed              | Soybean meal, heated   | 186     | .28         | .15          | 103 $\pm$ 34 | 28.5 $\pm$ 6.5   |
|                  | Soybean meal, unheated | 128     | .18         | .14          | 88 $\pm$ 19  | 16.3 $\pm$ 5.3   |

\* Total bile acids expressed as mg of chenodeoxycholic acid/ml of bile.

† Values  $\pm$  S.E. Average of 3 replicates of 9 to 12 chicks.

bile. Although there was considerably more variation in concentration of bile acids in bile taken 5 hours after feeding than in fasted chicks, there was no significant difference in bile concentration resulting from the 2 dietary treatments.

*Discussion.* These data show that the poor absorption of fat when young chicks were fed diets containing unheated soybean protein could be corrected by including the bile salt, sodium taurocholate, in the diet. Since soybean lecithin also was at least partially effective, the effect of the bile salt was probably one of increased "solubilization" of the fat in the intestinal tract of the chick. However, it seems unlikely that the dietary bile salt was helpful because of a deficiency of bile in chicks receiving the unheated soybean protein.

Garrett and Young(10) showed that chickens with bile fistulas still absorbed 40 to 50% of dietary fat supplied as safflower oil or olive oil. In most of our experiments with chicks receiving unheated soybean proteins, fat absorption values have normally been between 20 and 40% compared to 80 and 90% in chicks fed comparable diets with heat-treated soybean proteins. Although the measurements taken of bile secretion in this experiment were only of bladder bile, and not of bile from the thoracic duct, the data certainly do not point to any marked changes in bile composition or secretion due to the dietary treatment. However, since actual bile flow measurements were not made, the possibility of decreased bile secretion by chicks receiving the diets containing unheated soybean meal cannot be completely eliminated.

Gall bladder emptying was not interfered with as visual observation of gall bladders of chicks from the dietary treatments shortly after feeding indicated that contraction of the bladder and expulsion of the bile had taken place.

Rather than correct a deficiency of bile salts brought about by consumption of unheated soybean proteins, the additional bile salts may be beneficial in some other manner. Possibly the component of the soybean responsible for decreased fat absorption acts on the absorptive surface of the epithelial cells to reduce effective absorptive area. The bile salt may cause greater solubilization of lipid and thus more efficient contact with the available absorptive surface. Alternatively, the soybean protein may interfere with the action of the bile salt in promoting micelle formation or solubilization necessary for efficient fat absorption. In either case, however, the effect of age of chick on susceptibility to the induced malabsorption of fat by unheated soybean protein observed in earlier experiments(2) is still not explained.

*Summary.* The reduction in ability to absorb fat by chicks receiving diets containing unheated soybean meal can be overcome by supplementing the diet with sodium taurocholate or soybean lecithin. Cystine and taurine supplementation of the diet was not beneficial to fat absorption in chicks fed unheated soybean meal. Composition and secretion of bile collected from the gall bladder was not affected by feeding unheated soybean protein.

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### Phosphorus in Cell Fractions of Skeletal Muscle from Mice with Hereditary Muscular Dystrophy.\* (30036)

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Phosphorus-containing substances play important roles in the metabolism and contraction of muscle. The concentrations of several of them were found in various types of muscular dystrophy to differ considerably from normal levels(1-5). Such studies usually were made on whole muscle. This report deals with the distribution of phosphorus in various forms among the subcellular fractions of skeletal muscle of mice with hereditary muscular dystrophy.

**Materials and methods.** Dystrophic mice and control littermates of strain 129/Re(6) and of the recently developed hybrid strain 129 RexC57BL/6J(7) were obtained from the Jackson Laboratory, Bar Harbor, Maine. The animals were sacrificed when about 2 months old. The muscles from the hind legs were removed, minced with scissors, and homogenized at 0°-4°C in a Kontes Duall tissue grinder with 10 volumes of 0.1 M KCl, 0.039 M borate buffer, pH 7.1. The homogenate then was fractionated by differential centrifugation into nuclei-myofibrils, mitochondria, microsomes, and supernatant according to the procedure of Winnick and Winnick(8). Each fraction was extracted by a modification of the Schneider method(9) as follows: The acid-soluble substances were extracted at 0°C, once with 5% (w/v)

HClO<sub>4</sub> and twice with 2% HClO<sub>4</sub>. Lipids were then removed at room temperature by treatment, twice with 95% ethanol and 3 times with a 3:1 mixture of ethanol and ether. From the air-dried residue nucleic acids were extracted twice for 20 minutes with 5% HClO<sub>4</sub> at 70°C; the residue was finally washed with 5% HClO<sub>4</sub> at room temperature. Phosphorus, after digestion with 10% HClO<sub>4</sub>, was determined by the method of Taussky and Shorr(10). The nucleic acids were analyzed by the orcinol and indole tests for ribose and deoxyribose, respectively, according to Ceriotti(11). These tests were calibrated in terms of phosphorus. The data were evaluated statistically by the "t" test (12).

**Results and discussion.** The results presented in the tables as mean values with standard deviations were obtained with mice of strain 129/Re. Increase or decrease, in the following discussion, means the change in values for the dystrophic mice as compared to those for the controls. Table I shows that total phosphorus was markedly increased in the nuclear-myofibrillar fraction and decreased in the supernatant. The increase in the former was so pronounced that it led to a net increase for the homogenate. (Values for the sum of all fractions checked well with those of the homogenates; usually they were somewhat lower since the various

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