tion of phenyl quinone into the abdominal cavity. Much work (including spinal animals) remains to be done in order to elucidate the mechanism of this syndrome.

*Discussion.* It is not known whether the "syndrome" is closely associated with pain and the method may also lack some of the critical requirements for an ideal analgesic-testing method as outlined by Goetzl *et al.* (3). However, it has been shown that the "syndrome" caused by a noxious stimulus exerted in the abdominal cavity can be specifically blocked by local anesthetics (administered locally) and by analgesics (administered systemically). Furthermore, there is a good correlation between clinical efficacy of a number of analgesics and their potency as established by this method. In addition, the activity ratios of several narcotic analgesics are in general agreement with the experimental results reported in the literature. Therefore, it seems that the present method would serve best in evaluating, with the greatest clinical predictability, analgesic potency in laboratory animals.

*Summary.* A simple method has been designed for evaluating both non-narcotic and narcotic analgesics. The method is based upon the specific antagonism of analgesics to the typical "syndrome" produced by intraperitoneal injection of 2-phenyl-1,4-benzoquinone in mice. The antagonism occurs at doses far below those producing depression or other toxic effects. There is also a good correlation between clinical efficacy of a number of analgesics and their relative potency as established by this method. The endpoint is sharp, and the method is simple and quantitative.

1. Vander Wende, C., and Margolin, S., *Fed. Proc.*, 1956, v15, 494.

2. Litchfield, Jr., J. T., and Wilcoxon, F., *J. Pharm. Exp. Ther.*, 1949, v96, 99.

3. Goetzl, F. R., Burrill, D. Y., and Ivy, A. C., *Quart. Bull. Northwestern Univ. Med. School*, 1943, v17, 280.

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## Purified Diets for Ruminants.* (23346)

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It is established that the greater proportion of the carbohydrates in the diet of ruminants is absorbed as lower fatty acids of which acetic, propionic and butyric predominate(1). It appears, moreover, that on a roughage diet little or no enzymatically digestible carbohydrate escapes ruminal fermentation to the volatile acids(2,3), and that acetic acid is a major tissue metabolite of ruminants(4,5). Thus, it can be reasoned that the peculiarity of ruminant nutrition in contrast to that of the simple-stomach animal may be in the kind of substrate in the diet serving as the primary source of energy rather than in terms of new nutritional entities.

*Procedure.*† The formulas of the experimental diets are presented in Table I. Diet 1 was formulated along the lines of purified diets used for small laboratory animals; diet 2 contained acetic, propionic and butyric acids to simulate the predominant volatile acid end products of ruminal fermentation on a forage diet(6); diet 3 contained cellulose to simulate a roughage diet. Thirteen lambs, 1 to 3 weeks old, were separated from their dams and placed on a regimen of fortified fresh cow's milk(7) administered *via* nippled bottles. At approximately 3 months of age, 12 of these lambs were allotted at random to the experimental diets in a randomized block design; the extra lamb was assigned arbitrarily

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TABLE I. Purified Diets Used in Investigation.

| Components | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|
| | | % | |
| Casein | 30 | 30 | 30 |
| Glucose* | 36 | 24.1 | 16 |
| Hydrogenated veg. oil† | 4 | 4 | 4 |
| Corn starch | 20 | | 20 |
| Cellulose† | | | 20 |
| Salts of lower fatty acids‡ | | 31.9 | |
| Vitamin mixture§ | 5 | 5 | 5 |
| Mineral mixture | 5 | 5¶ | 5‖ |

* Crystalline glucose, Corn Products Sales Co., Norfolk, Va.

† Grateful acknowledgment is made to Buckeye Cotton Oil Co., Cincinnati, Ohio, for Primex B and C (pure vegetable shortening) and alpha cellulose cotton linter pulp.

‡ Ca propionate 4.8%, Na propionate 3%, Na acetate 9%, K acetate 8.5%, Mg acetate 3%, and Na butyrate 3.6%. Grateful acknowledgment is made to E. I. duPont de Nemours and Co., Wilmington, Del., for Ca and Na propionate and to Farmers Cooperative Exchange, Raleigh, N. C., for acetate and butyrate salts.

§ Vit. mixture/100 lb diet: thiamine HCl, 400 mg; riboflavin, 850 mg; nicotinic acid, 1.13 g; Ca pantothenate, 1.42 g; pyridoxine HCl, 570 mg; folic acid, 57 mg; p-aminobenzoic acid, 1.13 g; inositol, 11.35 g; biotin, 11.4 mg; choline chloride, 113.45 g; menadione (2-methylnaphthoquinone), 115 mg; 0.1% vit. $B_{12}$ (with mannitol), 4.66 g; alpha tocopherol acetate, 570 mg; glucose, 2132 g. Acknowledgment is gratefully made to Merck and Co. for contributing the vitamins.

Admin. 4000 I.U. A and 400 I.U. D/day/100 lb body wt *via* capsules generously contributed by R. P. Scherer Corp., Detroit, Mich.

‖ Mineral mixture/100 lb diet: $CaHPO_4$, 818 g; KCl, 273 g; NaCl, 239 g; $MgSO_4$, 204 g; $CuSO_4 \cdot 5H_2O$, 893 mg; $FeSO_4 \cdot 2H_2O$, 7648 mg; $MnSO_4 \cdot H_2O$, 1399 mg; ZnO, 2263 mg; $CoCO_3$, 9 mg; glucose, 722 g.

¶ Mineral mixture/100 lb diet: trace minerals, Cu, Fe, Mn, Zn and Co, 12 g (See ‖); $NH_4Cl$, 138 g; $H_3PO_4$ (85%), 226 g; glucose, 1892 g.

to diet 2. The change-over from the basal milk diet to the dry experimental diets was effected in approximately 3 weeks. The lambs were fed individually in pens made of aluminum pipe siding and wooden slatted floors. Lambs receiving diets 1 and 3 were fed *ad libitum*, whereas those receiving diet 2, because of its deliquescent nature, were restricted to the amounts they would consume 3 times a day. The animals were weighed at weekly intervals. Blood samples were taken biweekly, before the morning feeding, from the jugular vein of the experimental animals. The serum of these samples was analyzed for calcium, phosphorus and magnesium by the methods used by Hawkins *et al.*(8) and iron and copper by the methods described by Matrone *et al.*(9). Hemoglobin was determined by the method of Shenk *et al.*(10) and blood glucose by the method of Mendel *et al.*(11).

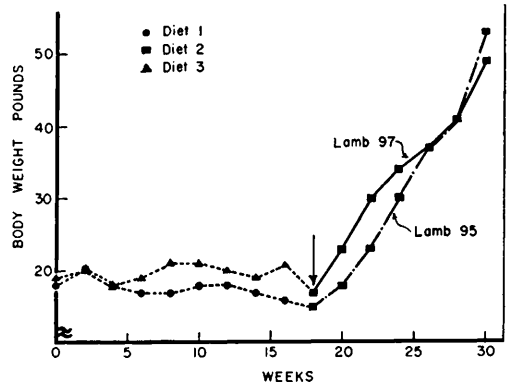


FIG. 1. Responses of 2 lambs to diets 1 and 3, followed by diet 2.

*Results.* After 18 weeks on experiment, only the lambs on diet 2 had made appreciable gains (Table II). In view of these results, one lamb on diet 1 and one lamb on diet 3 were changed to diet 2. The response of these animals to diet 2 (Fig. 1) was marked. The average daily gain for these 2 lambs over a 12-week period was 0.41 lb, and the average feed efficiency 3.1 lb of feed/lb of gain.

The 40-week gains of the animals continued on original treatments are shown in Table II. It is apparent that the superior response of lambs receiving diet 2 over those receiving either 1 or 3 was maintained. These data also indicate that the lambs fed diet 3 gained more than those fed diet 1. Feed intake paralleled the gains, *e.g.*, during the last 15 weeks of the 40-week experimental period the aver-

TABLE II. Averages of Initial Weights, 18-Week Gains and 40-Week Gains of Lambs on 3 Purified Diets.

| Diet No. | Initial wt, lb | 18-wk gain, lb | 40-wk gain, lb |
|---|---|---|---|
| 1 | 26 | .5 (4)* | 6.7 (3)† |
| 2 | 20 | 27.0 (5) | 68.5 (4)‡ |
| 3 | 20 | 2.0 (4) | 18.3 (3)† |

* No. in parentheses = No. of lambs receiving diet.

† One lamb changed to diet 2 (Fig. 1).

‡ One animal died; autopsy revealed gastro-enteritis and extensive passive congestion of both lungs.

age daily intake per lamb was as follows: diet 1, 0.64 lb; diet 2, 1.42 lb, and diet 3, 0.93 lb.

Other differences were also observed. Lambs on diets 1 and 3 ate their own wool in characteristic patterns determined by their ability to reach certain areas of their bodies, whereas the lambs on diet 2 were not observed either eating their own wool or with areas denuded of wool. It was observed, moreover, that the lambs on diet 2 appeared to have a higher than normal respiration rate, which was accentuated on hot days. This effect might have been a contributing factor in the death, during the summer, of the most rapidly growing lamb. Autopsy revealed gastro-enteritis and extensive passive congestion of both lungs. Several times thereafter during the summer, when other lambs on diet 2 developed an excessive rate of respiration, it was reduced by spraying them with water.

The mineral constituents of the serum (calcium, magnesium, phosphorus, iron and copper) and hemoglobin of the blood of all of the experimental animals were within the normal range. Glucose levels of the blood, which averaged $42.6 \pm 10.5$, $41.6 \pm 10.8$, and $44.6 \pm 11.2$ mg % for the lambs receiving diets 1, 2 and 3, respectively, were not significantly different.

*Discussion.* The data that have been presented strengthen the view that the volatile fatty acids play a vital role in ruminant nutrition. The results with growing lambs in this investigation are in accord with supplementary observations with 3 mature sheep, in which weight changes during a 4-month period were as follows: diet 1, –50 lb; diet 2, +11 lb, and diet 3, –16 lb. The higher respiration rate observed in the animals fed the diet containing the salts of the volatile fatty acids may possibly be the result of the higher specific dynamic action associated with the metabolism of acetate(3). Another possibility is that a "subclinical" alkalosis resulted from the high cation content of the diet.

The poor growth of the animals receiving diet 1 was probably due, in part at least, to the changes that occurred in the dietary starch and glucose in the rumen rather than to the inadequacy of the diet. Except for a lower percentage of fat, diet 1 is quite similar to synthetic milk diets used successfully with young calves by Wiese *et al.*(12) and Blaxter (13). Under our conditions, moreover, lambs fed a fortified milk diet(7) grew in an apparently normal manner to a weight of 80-90 lb. In the experiments cited, however, the diets were fed in liquid form *via* nipples. In such a procedure, presumably the diet bypasses the rumen to a considerable extent and thereby escapes ruminal fermentation. When the diet is fed in the dry form, most of it would be expected to enter the rumen, where the dietary starch and glucose are fermented, probably to lactic acid(1). Although under normal circumstances lactic acid in the rumen is unstable and is fermented further to propionic and acetic acids(1), under heavy starch feeding the lactate may remain in the rumen in considerable concentrations and the pH then may become very acid(14). It is possible that such a sequence of action took place in the rumen of the animals fed diet 1, since its content of starch and glucose is high.

The growth obtained with the lambs fed the cellulose diet was below expectation. One possible explanation of this result may be that the inoculum for a normal rumen flora was absent, since the animals were housed away from older ruminants and had access only to purified ingredients. The study with mature sheep, in which the animal on diet 2 gained 11 lb whereas the animal on diet 3 lost 16 lb, suggests that other factors were also involved, since presumably the sheep started with a normal rumen flora.

*Summary.* The efficacy of 3 purified diets, each containing a different mixture of energy-yielding constituents, for growth of lambs was investigated. Preliminary results indicate that lambs fed a diet containing glucose and 31.9% of the salts of volatile acids, acetic, propionic and butyric, performed better than lambs on diets containing either starch and glucose or starch, glucose and cellulose.

1. Elsden, S. R., and Phillipson, A. T., *Ann. Rev. Biochem.*, 1948, v17, 705.

2. Heald, P. J., *Brit. J. Nutr.*, 1951, v5, 75.

3. McClymont, G. L., *Aust. J. Agr. Res.*, 1951, v2, 158.

4. Reid, R. L., *ibid.*, 1950, v1, 338.

5. McClymont, G. L., *Aust. J. Sci. Res.*, Series B, Biol. Sci., 1952, v5, 374.

6. Kiddle, P., Marshall, R. A., and Phillipson, A. T., *J. Physiol.*, 1951, v113, 207.

7. Hartman, R. H., Matrone, G., and Wise, G. H., *J. Nutrition,* 1955, v57, 429.

8. Hawkins, G. E., Jr., Wise, G. H., Matrone, G., Waugh, R. K., and Lott, W. L., *J. Dairy Sci.*, 1955, v38, 536.

9. Matrone, G., Peterson, W. J., Baxley, H. M., and Grinnells, C. D., *ibid.*, 1947, v30, 121.

10. Shenk, J. H., Hall, J. L., and King, H. H., *J. Biol. Chem.*, 1934, v105, 741.

11. Mendel, B., Kemp, A., and Myers, D. K., *Biochem. J.*, 1954, v56, 639.

12. Wiese, A. C., Johnson, B. C., and Nevens, W. B., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 521.

13. Blaxter, K. L., Rook, J. A. F., and MacDonald, A. M., *J. Comp. Path.*, 1954, v64, 157.

14. Cuthbertson, D. P., and Phillipson, A. T., *Biochemistry of Physiology and Nutrition,* 1953, vII, p146, Academic Press, N. Y.

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## Effect of Ferric Chloride on Mobilization of Accumulated Liver Cholesterol, in Mice.* (23347)

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Siperstein, Nichols, and Chaikoff(1) successfully prevented hypercholesterolemia in cockerels by addition of ferric chloride to a diet rich in bile concentrate and cholesterol. They based their work on two well-known facts: 1. Cholesterol absorption in the intestinal tract of the rat is hindered if bile is not present(2); and 2. Ferric chloride precipitates bile salts, *in vitro*. In our previous experiment(3), addition of varying amounts of ferric chloride to cholesterol-rich diets did not prevent the accumulation of exogenous cholesterol in mouse liver. Friedman, Byers, and Gunning(4) showed that in rats excess organ cholesterol is converted to bile acids prior to excretion. It has been shown by Hummel(5) that the mobilization of excess liver cholesterol in mice is prevented by dietary bile acids. It would follow from these observations that any substance which would remove bile acids from the intestinal tract, should increase the rate of cholesterol mobilization. This experiment attempts to answer 2 questions: Does the addition of ferric ions to the diet increase rate of liver cholesterol mobilization in mice with elevated liver cholesterol? Do ferric ions reverse effects of dietary cholic acid?

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*Methods.* Approximately 200 albino female Webster strain mice, weighing 15-20 g, were placed on a cholesterol-free diet (C.F.D.) for 2 weeks. The animals were then divided into 2 groups: Group A with 40 mice, and Group B with 160 mice. Group A was continued on the basic C.F.D. while Group B diet was supplemented with 1% cholesterol and 0.5% cholic acid. After a 3-week period on this regime, 7 Group A and 10 Group B animals were sacrificed. As shown in Table I, the supplemented diet resulted in a significant build-up of liver cholesterol in the Group B animals, but in no change in either total lipids or phospholipids. After this build-up period, the remaining Group B animals were divided into 4 groups of approximately 40 mice each and placed on the following diets: Group C received the C.F.D.; Group D received the C.F.D. plus 0.5% cholic acid; Group E, C.F.D. plus 1% ferric ions; and Group F, C.F.D. plus 0.5% cholic acid and 1% ferric ions. At weekly intervals about one-fourth of the mice from each group were sacrificed by decapitation. The livers were immediately excised, drained of excess blood, weighed, and quick frozen. The livers were dried over phosphorous pentoxide, weighed, and extracted with a 1:1 mixture of methanol and chloroform. Aliquots of the extracts were