increased the rate of gain. A variable accumulation of hair in the stomach was also observed in the present study for stock pairs fed purified diets. Further work is needed to determine the value of adding cellulose to the ration on the reproduction performance.

The weight of the young at weaning for both sexes was similar, *i.e.* 20.5 for 32 males and 21.0 for 44 females from a pair fed stock ration. It can be seen from Table II that the weaning weights of young were similar for the groups receiving different diets. This observation cannot be accurately evaluated, however, since smaller litters were weaned by the groups fed purified diets, and thus would result in a variable stress of lactation.

It is recognized that the purified diets used favor the development of dental caries and this may have affected the reproduction performance. The animals ate the rations readily, however, and reached a mature weight approximating that obtained when stock ration was fed. A few pairs were fed starch as the carbohydrate, but their performance did not appear to be improved over that for the groups fed sucrose diets.

Summary. The cotton rat (*Sigmodon hispidus hispidus*) was shown not to require a dietary source of biotin or pteroylglutamic acid for optimum growth when a purified ration was fed. The growth response obtained with nicotinic acid supplementation was dependent on the casein (tryptophan) content of the diet in that as the casein level was increased the growth response attributable to the addition of nicotinic acid was decreased. A poor reproduction performance was observed when purified diets were fed. Improvement was noted when liver extract was fed in addition to the purified diet or when stock ration was fed.

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Pyridoxine, Choline and l-Methionine Content of Parenteral Liver Extract.

GUY W. CLARK.

From Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

In the nonspecific use of parenteral liver extract, the physician is not much interested in a statement of antianemia potency as he is in knowing the amounts of the several biologically active substances (factors of the B complex) present. Numerous requests for such information have made it necessary to analyze the various types of liver extract. The advent of the several microbiological assays made it possible to determine the amounts of several of the B factors. A previous article[1] contains data for total solids, total nitrogen, riboflavin, niacin, pantothenic acid, and folic acid (*L. casei factor*) present in several samples of refined, concentrated extracts and in the dilute, crude preparations. In this brief paper, data is presented for the pyridoxine, choline, and *l*-methionine content of twelve different commercial extracts.

Materials and Methods. The liver extracts, conforming to U.S.P. standards, included in this report represent random samples purchased from a wholesale druggist and unselected samples of our own products. The total amounts of pyridoxine, choline, and *l*-methionine extracted from whole liver by hot, acidified water (pH 5.0—85°C) are shown, numbers 34 and 35 in Table I. The method of Hochberg, Melnick, and Oser[2] has been used for the determination of pyridoxine. The microbiological method described by Stokes, *et al.*[3] has been used for the estimation of *l*-methionine. Choline has

1 Clark, G. W., *Am. J. Med. Sci.*, 1945, **209**, 520.

2 Hochberg, M., Melnick, D., and Oser, B. L., *J. Biol. Chem.*, 1944, **155**, 119.

3 Stokes, J. L., Gunness, M., Dwyer, I. M., and Coswell, M. C., *J. Biol. Chem.*, 1945, **160**, 35.

TABLE I.
Vitamin Content of Liver Extracts for Parenteral Use II. A Comparison of Crude and Concentrated Preparations.

Extract No.	U.S.P. units per cc	Pyridoxine HCl		Methionine HCl		Choline chloride	
		Per cc, μg	Per unit, μg	Per cc, mg	Per unit, μg	Per cc, mg	Per unit, μg
22	15	7.9	0.5	3.1	207	8.9	593
23	15	3.4	0.2	3.4	227	7.0	467
24	15	21.0	1.4	1.2	80	1.1	73
25	15	5.9	0.4	2.9	193	1.1	73
26	15	6.5	0.4	1.8	120	0.6	43
27	10	12.4	1.2	1.3	130	3.5	350
28	4	8.8	2.2	1.7	425	10.3	2575
29	3.3	8.5	2.6	2.2	667	14.0	4242
30	2	5.6	2.8	0.6	300	4.6	2300
31	2	5.7	2.8	2.5	1250	3.9	1950
32	2	9.7	4.8	3.7	1850	13.4	6700
33	2	4.7	2.3	1.8	900	13.7	6850
Mean of 12 samples		8.3	1.8	2.2	529	6.8	2184
" " 5-15 U samples		8.9	0.6	2.5	165	3.7	250
" " 4-2 U "		6.4	3.2	2.2	1075	8.4	3875
34*		189.4‡		23.0‡		223.2‡	
35†		151.8‡		29.8‡		198.1‡	

* Unfractionated crude concentrate obtained by hot water (pH 5.0—85°C) extraction of beef liver.

† Unfractionated crude concentrate obtained by hot water (pH 5.0—85°C) extraction of pork liver (90% pork—10% beef).

‡ Amount present in crude concentrate obtained from 100 g of liver.

been determined by the neurospora method of Horowitz and Beadle.[4]

Discussion. As previously reported[1] for riboflavin, niacin, pantothenic acid, and folic acid (*L. casei factor*), and as can be seen from Table I, the various extracts show considerable variation in their content of pyridoxine, choline, and *l*-methionine. Before discussing the parenteral liver extracts it might be well to see how much pyridoxine, choline, and *l*-methionine is extracted from whole liver by the usual hot (85°C.), acid-water (pH 5.0) treatment. The values for unfractionated beef liver concentrate (34) and unfractionated pork liver concentrate (35) are shown at the bottom of Table I. Using the average for both beef and pork, we obtain 189 μg of pyridoxine, 223 mg of choline and 23 mg *l*-methionine from 100 g of whole liver. Using these values as a "yardstick," it becomes evident that the addition of ethyl alcohol to make 65-70%, by volume, together with the other procedures used in the preparation of suitable parenteral extracts, removes a large part of each of the several B factors. Without definite knowledge as to the amount of liver used in the preparation of various extracts, it is not possible to estimate the percentage of the various factors present in the finished preparations. The data presented in Table I shows the amount of each of the 3 substances per cc and per U.S.P. injectable unit. In the nonspecfiic uses of liver extract very little attention is paid to the antianemia potency; therefore, the best evaluation of the different samples and types of liver extract is the comparison of the amount of each factor present in 1 cc.

Using the average value of 5 concentrated extracts, the pyridoxine present per cc is approximately 5% of that originally present in the crude concentrates, 34 and 35; the choline is less than 2%; the *l*-methionine is about 9%. Using the average value of 4 crude extracts, the pyridoxine per cc is about 4%; the choline 4%; and the *l*-methionine 8%. Actually on a cc basis, there is no significant difference between the pyridoxine and *l*-meth-

[4] Horowitz, N. H., and Beadle, G. N., *J. Biol. Chem.*, 1943, **150**, 325.

ionine content of the refined, concentrated extracts and the dilute, crude preparations. There is definitely more choline in the crude extracts.

Since we are dealing with the extraction of preformed substances stored in the liver and since nutrition of the animal affects the storage of such substances, it is obvious that the data presented are relative rather than absolute.

Summary. From further quantitative studies of U.S.P. refined, concentrated and from dilute, crude liver extracts, it is evident, using average values per cc, that the amounts of pyridoxine and *l*-methionine are approximately the same in the two types of extract. The crude extracts contain more choline than the refined preparations.

The writer is indebted to Alice Craig and Maurice Avery for the analytical data presented.

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A Differential Response of the Rodent Adrenal Gland to Acute Starvation.

SAVINO A. D'ANGELO, ALBERT S. GORDON, AND HARRY A. CHARIPPER.

From the Department of Biology, Washington Square College of Arts and Science, New York University.

It has been shown that the adrenal gland of the female guinea pig progressively enlarges during starvation and that this hypertrophy requires the presence of an intact hypophysis and cannot be appreciably overcome, in the starved animal, with cortical hormone overdosage.[1] The experiments of Cameron and Carmichael[2] have indicated that enlargement of the adrenals in the rat occurs only in the advanced stages of acute starvation, and that this increase in size is not a true physiological growth but is rather a pathologic change involving increased water content. The present report describes preliminary experiments on rats and guinea pigs which demonstrate differences in adrenal response to acute inanition.

Materials and Methods: Female guinea pigs (290-320 g) and adult rats of both sexes (150-250 g) were subjected to acute starvation over a period of 6-11 days by complete food deprivation (water allowed freely). During starvation some animals were given daily subcutaneous injections of 5-18 mg of desoxycorticosterone acetate (DOCA) in saline or in sesame oil.* Starved controls received injections of saline or sesame oil alone or remained untreated. Corresponding groups of animals received similar experimental treatment and the complete normal laboratory ration. All animals were exsanguinated 24-36 hours after the last injection, or sooner, if moribund. The adrenals were removed, trimmed, weighed in the fresh state, and dried for 24-36 hours at 100-105° C to constant weight for solid and water content.

Results. The data (tables) indicate that the morphologic response of the adrenals to acute starvation is different in the guinea pig and the rat. In the former, the adrenal gland shows an absolute increase in size and in solid content with advanced starvation. The water content of these hypertrophied glands increases proportionately, and the percentages of solids and water are not appreciably changed from the normal. In contrast, the adrenals of acutely starved rats often fail to enlarge or may diminish in size. Likewise, the solid content of the gland does not increase but either remains unchanged or may

[1] D'Angelo, S. A., Gordon, A. S., and Charipper, H. A., *Endocrinology*, 1948, **42**, 399.

[2] Cameron, A. T., and Carmichael, J., *Canad. Research*, 1946, **24**, 37.

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