from 0.2 to 1.5 g. The injection of control untreated amino acids was ineffective in 4 dogs even after the injection of 20 g in 120 minutes.

In 12 dogs which were on the control diet containing untreated flour (*i.e.*, unbleached flour), the intravenous administration of the agene-treated amino acid mixture was followed by seizure patterns in the electroencephalogram of 4 dogs, marked abnormalities in 3 dogs, and no significant change in 5 dogs. In the animals with abnormal E.E.G.'s produced by intravenously administered agenized amino acids, the intracisternal injection of 1 to 2 cc of the NCl_3-treated amino acid mixture was followed by seizures within 5 minutes after injection.

Experiments have been conducted with individual amino acids treated in their crystalline states and the results have been negative for arginine, glutamic acid, glycine, histidine, lysine, methionine, tryptophane and tyrosine; equivocal for proline, serine, and aspartic acid; and positive for cysteine hydrochloride. A possibility that should not be overlooked is that the toxic compound in foodstuffs may be an altered polypeptide.

Summary. When an amino acid mixture approximating the composition of gliadin is treated with nitrogen trichloride gas (NCl_3) this mixture is so altered as to make it convulsant for dogs when given intravenously. The convulsant activity is most readily demonstrated in dogs which have been on a bleached (agenized) flour diet for 5 to 7 days, presumably because these animals have accumulated sufficient quantity of the toxic material *via* the alimentary tract, to make them highly susceptible to an additional increment received *via* the blood stream.

16108

Adrenal Cortical Activity in Urine of Horses.

EDWIN A. RISLEY, ANN B. SCHULTZ, WILLIAM B. RAYMOND, AND RICHARD H. BARNES.

From the Department of Biochemistry, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

The presence of a substance in urine that protects adrenalectomized rats from the stress of cold was first noted by Anderson, Haymaker and Joseph[1] and Weil and Browne.[2] More extensive investigations have shown that extracts of urine from normal human subjects when tested in adrenalectomized rats cause an elevation of liver glycogen,[3,4] increased life maintenance,[3,5] prolongation of muscle work performance,[6] and prevention of water intoxication.[7] Although the above studies have employed extracts of human urine, Selye and Dosne[8] have reported that the urine of large domestic animals contains cortin-like activity. The purpose of this in-

1 Anderson, E., Haymaker, W., and Joseph, M., *Endocrinology*, 1938, **23**, 398.

2 Weil, P., and Browne, J. S. L., *Science*, 1939, **90**, 445.

3 Venning, E. H., Hoffman, M. M., and Browne, J. S. L., *J. Biol. Chem.*, 1943, **148**, 455.

4 Horwitt, B. N., and Dorfman, R. I., *Science*, 1943, **97**, 337.

5 Dorfman, R. I., and Horwitt, B. N., *Fed. Proc.*, 1943, **2**, 60.

6 Shipley, R. A., Dorfman, R. I., and Horwitt, B. N., *Am. J. Physiol.*, 1943, **139**, 742.

7 Schiller, S., and Dorfman, R. I., *Endocrinology*, 1943, **33**, 402.

8 Selye, H., and Dosne, C., *Lancet*, 1940, **239**, 70.

TABLE I.
Liver Glycogen Response of Horse Urine Extracts.

Sample No.	Avg urine equiv. cc	Liver glycogen: Negative controls mg/100 g liver weight	Liver glycogen: Urine extract mg/100 g liver weight	Percentage increase %	Remarks
		Total extracts.			
B-18	200	37 (40)*	44 (22)*	+19	Normal urine
38-191	"	33 (35)	38 (19)	+15	Pregnant mare
176-10	"	45 (19)	53 (10)	+18	" "
		Ketonic fraction			
38-174	400	40 (12)	80 (9)	+100	Normal urine
38-175-2	"	80 (10)	90 (10)	+ 12	" "
38-187	250	130 (15)	190 (10)	+ 46	" "
38-191-1	330	30 (20)	100 (15)	+230	Pregnant mare
176-5-1	250	30 (10)	80 (10)	+170	" "
		Non-ketonic fraction.			
38-174	200	60 (10)	60 (3)	0	Normal horse
38-175-2	"	90 (15)	60 (9)	—33	" "
38-187	"	90 (15)	50 (9)	—45	" "
38-191	"	30 (15)	30 (9)	0	Pregnant mare

* Numbers in parentheses refer to number of mice employed.

vestigation was to assess the concentration of cortin-like activity in the urine of horses.

Method. The assay employed was a modification of the methods used by Venning, *et al.*[9] and Eggleston, *et al.*[10] Male white mice of the Swiss-Webster strain from our own stock colony weighing 20-25 g were used. At least a week before being used the mice were placed on a purified diet which contained 25% protein, 48% carbohydrate, 25% fat and 2% salt mixture,[11] plus adequate vitamin supplements. The animals were anesthetized with ether and adrenalectomized by the usual lumbar route. Following adrenalectomy they were placed in a warm room and given the above diet and 0.9% NaCl in place of their usual drinking water. On the morning of the fourth postoperative day food was removed and each mouse was given 25 mg glucose orally, and the first of 7 hourly injections of the extract under test. One hour after the last injection the mice were weighed and anesthetized with nembutal. The livers were quickly removed, weighed and placed in hot 30% KOH for digestion. Glycogen was precipitated with alcohol and after hydrolysis, total reducing substances were determined by the method of Good, Kramer and Somogyi.[12] Total liver glycogen has been expressed as milligrams of glucose per 100 g of liver weight.

Urine Extracts. Urine was collected under toluene and acidified with acetic acid. All urines were extracted 3 times with one-quarter volume of ethylene dichloride or chloroform, usually employing a motor stirrer for the extraction. The combined extracts were then evaporated almost to dryness under reduced pressure and taken up in small amounts of 95% ethanol and diluted for injection or further fractionated by the method described by Venning, *et al.*[13]

Results. Table I lists the results obtained with 3 different types of extracts. Crude urine extracts that were not fractionated are referred to as total extracts. The ketonic and non-ketonic fractions were obtained by the Girard's reagent separation procedure.[13] Many of the total extracts of horse urine that have been prepared in this laboratory have shown evidence of toxic substances that resulted in a decrease in liver glycogen below the level of the negative controls. Dorfman,

9 Venning, E. H., Kazmin, V. E., and Bell, J. C., *Endocrinology*, 1946, **38**, 79.

10 Eggleston, N. M., Johnston, B. J., and Dobriner, K., *Endocrinology*, 1946, **38**, 197.

11 Bosshardt, D. K., Ciereszko, L. S., Buffington, A. C., and Arnow, L. E., *Arch. Biochem.*, 1945, **7**, 1.

12 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

13 Venning, E. H., Hoffman, M. M., and Browne, J. S. L., *Endocrinology*, 1944, **35**, 49.

et al.[14] Venning, *et al.*[3] have commented on this toxic effect found in extracts of human urine. Fractionation evidently removes some of the toxic activity since glycogen responses are higher in the ketonic fraction than in the total extracts. As was to be expected no evidence of cortin-like activity was found in the non-ketonic fractions.

As a standard preparation for comparison, Wilson's aqueous adrenal cortical extract has been used. Comparing liver glycogen responses to Wilson's extract with the average response of the ketonic fraction of the horse urine extracts it was calculated that 1 liter of normal horse urine contains an equivalent of 0.10-0.15 cc of Wilson's extract and 1 liter of pregnant mare urine contains an equivalent of 0.30-0.40 cc of this extract. These figures compare favorably with results of Dorfman *et al.*[15] and Venning *et al.*[3] on human urine extracts but appear to be definitely lower than the results reported for horse urine by Selye and Dosne.[8] The quantitative assay of the concentration of cortin-like material in urine is complicated by so many factors that the results presented here should be considered only as estimations.

Summary. The presence of cortin-like activity in extracts of horse urine has been confirmed. It is estimated that the concentration of this active material is approximately the same in urine of normal horses and normal humans. There is some indication that urinary cortin-like activity is increased in pregnancy.

14 Dorfman, R. I., Shipley, R. A., Schiller, S., and Horwitt, B. N., *Endocrinology*, 1946, **38**, 165.

15 Dorfman, R. I., Horwitt, B. N., and Fish, W. R., *Science*, 1942, **96**, 496.

16109

Nutrition of the Mouse. IV. Comparison of Bacterial Population of Two Highly Inbred Strains.*

LORRAINE S. GALL, PAUL F. FENTON, AND GEORGE R. COWGILL.

From the Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University, New Haven, Conn.

It was shown by Rogers, McElroy and Cowgill[1] that the nutritional requirements for reproduction and lactation differed for 2 strains of mice. Fenton and Cowgill[2] showed the existence of a strain difference with respect to the riboflavin requirement for growth. Unpublished work in this laboratory has shown that this is also true of the pantothenic acid requirement. This dissimilarity of the nutritional requirements of 2 strains of mice may in part be due to different rates of intestinal synthesis of essential nutrients. The cecum has been thought to be an important site of the synthesis of B vitamins.[3,4,5,6] Man-

* Supported in part by the Nutrition Research Fund of this laboratory and in part by a grant from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council. The early studies were supported by grants from the Nutrition Foundation, Inc., and the Anna Fuller Fund.

We wish to acknowledge gifts of B-complex vitamins from Merck and Company, Inc., Hoffmann-LaRoche, Inc., and Lederle Laboratories, Inc. The technical assistance rendered by Marie A. Stone was greatly appreciated.

1 Rogers, L. K., McElroy, L. W., and Cowgill, G. R., *Science*, 1942, **95**, 203.

2 Fenton, P. F., and Cowgill, G. R., *J. Nutrition*, 1947, **34**, 273.

3 Guerrant, N. B., Dutcher, R. A., and Tomey, L. F., *J. Biol. Chem.*, 1935, **110**, 233.

4 Taylor, A., Pennington, D., and Thacker, J., *U. of Texas Pub.*, 1942, **4237**, 135.

5 Schweigert, B. S., McIntire, J. M., Henderson, L. M., and Elvehjem, C. A., *Arch. Biochem.*, 1945, **6**, 403.

6 Mannering, G. J., Orsini, D., and Elvehjem, C. A., *J. Nutrition*, 1944, **28**, 141.