factors than the total number of amino acid residues or content of primary amide groups. Alteration in the sequence of the amino acids in the peptide chains is a possibility which should be considered in understanding this interesting problem.

Summary. The hemoglobin from sickle cell anemic individuals does not differ significantly in primary amide content from normal hemoglobin. Normal human hemoglobin was found to contain 26 primary amide groups per mole.

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Choline Requirement of Rats as Influenced by Age, Strain, Vitamin B_{12} and Folacin.* (18873)

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It was shown(1-3) that there was considerable variation in the minimum choline requirement of rats from different strains, sex, litter, and age. Copeland(3) noted that hooded rats of the Alabama Experiment Station strain (AES) had a much higher choline requirement for protection against renal hemorrhage than did albino rats of the Wistar strain. Since rats of the AES strain develop a high incidence of neoplasms(4-6) when subjected to chronic choline deficiency, it was of interest to investigate the choline requirement of the albino Sprague-Dawley (SD) strain as compared to the AES strain. The reports(7-9) that young rats of the Wistar strain are capable of synthesizing sufficient methyl and choline or of utilizing methanol for choline synthesis for growth and protection against renal damage prompted the investigation as to whether age and strain difference would influence these mechanisms under the experimental conditions employed in this laboratory.

Experimental. Weanling rats (21 days) of the SD and the AES strains weighing 40-45 g were placed in individual cages and uniformly grouped with respect to number, weight, sex, and litter. Feed and water were supplied *ad lib.* The basal diet contained extracted peanut meal 30 (50% protein)(10), sucrose, 39.5, extracted casein 6(11), salt mixture (undried) 4.4(11), L-cystine 0.1, cod liver oil 1, and lard 19. Vitamins were added (mg per kg of diet) as follows: Thiamine 2, pyridoxine 2, riboflavin 4, calcium pantothenate 10, niacin 20, i-inositol 200, alpha-tocopherol 25, alpha-tocopherol acetate 25, and 2-

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1. Engel, R. W., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 193.

2. Engel, R. W., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 281.

3. Copeland, D. H., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 33.

4. Copeland, D. H., and Salmon, W. D., *Am. J. Path.*, 1946, v22, 1059.

5. Engel, R. W., Copeland, D. H., and Salmon, W. D., *Ann. New York Acad. Sci.*, 1947, v49, 49.

6. Schaefer, A. E., Copeland, D. H., Salmon, W. D., and Hale, O. M., *Cancer Res.*, 1950, v10, 786.

7. Stekol, J. A., and Weiss, K., *J. Biol. Chem.*, 1950, v186, 343.

8. du Vigneaud, V., Ressler, C., and Rachele, J. R., *Science*, 1950, v112, 267.

9. Arnstein, H. R. V., *Biochem. J.*, 1951, v48, 27.

10. Engel, R. W., *J. Nutrition*, 1948, v36, 739.

11. Salmon, W. D., *J. Nutrition*, 1947, v33, 155.

12. Engel, R. W., *J. Biol. Chem.*, 1942, v144, 701.

TABLE I. Choline Requirement of Sprague-Dawley Rats for Protection Against Hemorrhagic Kidneys and for Lipotropism as Influenced by Vit. B_{12} and Folacin (8 Rats per Treatment).

Dietary supplement				Avg liver analyses		
Choline, %	Vit. B_{12}* and folacin	Avg wt gain, g/2 wk	Incidence of renal damage, %	Fat dry basis, %	Moisture, %	Choline per g dry liver, mg
.04	0	35	63	55.3	53.1	3.2
.08	0	58	25	52.3	52.4	3.9
.10	0	69	0	40.3	59.7	4.3
.20	0	73	0	19.5	66	5.8
.30	0	68	0	15.8	67.8	7.2
.40	0	75	0	12.9	67.8	7.8
.04	+	71	0	55.2	52.5	3.7
.08	+	75	0	43.3	61.3	4.3
.10	+	70	0	33.3	62.6	6.4
.15	+	75	0	15.3	67.9	7.7
.20	+	81	0	12.8	69	8.5

* Vit. $B_{12} = 30$ μg, and folacin = 2 mg/kg diet.

methyl-1,4-naphthoquinone 5. Where indicated, folacin was added at 2 or 10 mg and vit. B_{12} at 30 or 150 μg per kg of diet. The total methionine and choline contents of the diet were 0.30% and 0.005%, respectively.

All rats were necropsied after death or at the end of the 14-day experimental period; kidneys were examined for gross pathological lesions; and livers were analyzed for moisture, fat, and choline. Liver fat was expressed as per cent total ether-extractable material on a dry weight basis. Choline was determined by the method of Engel(12) with the following modifications: The sample was continuously extracted for 48 hours with boiling methanol rather than the 3 separate extractions totaling 24 hours; normal propyl alcohol was substituted for ethyl alcohol as a wash solution for the choline reineckate.

Results. The results of supplementing the basal diet for the SD strain of rats with varying levels of choline chloride with and without vit. B_{12} and folacin are given in Table I. Supplementation of the diet with vit. B_{12} and folacin markedly suppressed the accumulation of fat in the livers, reduced the incidence of renal damage, and increased the choline content of the livers. The liver fat of the rats fed the basal diet supplemented with 0.15% and 0.20% choline chloride plus vit. B_{12} and folacin was equal to that of rats receiving 0.30% and 0.40% choline chloride respectively, without vit. B_{12} and folacin. In the absence of dietary vit. B_{12} and folacin, the incidence of kidney hemorrhage was 67% and 25% when the choline chloride level was 0.04% and 0.08%, respectively. No renal damage occurred when the diet was supplemented with 0.04% choline chloride, and vit. B_{12} and folacin.

The choline concentration of the livers of rats receiving 0.10% choline chloride was increased an average of 49% (*i.e.*, from 4.3 to 6.4 mg per g dry liver) by the additional dietary supplement of vit. B_{12} and folacin.

The influence of age, strain, and dietary methanol supplements on the pathology of acute choline deficiency in the presence of adequate vit. B_{12} and folacin is shown by the data in Table II. Under these experimental conditions, both strains of rats failed to develop hemorrhagic kidneys when they were maintained on an adequate stock diet until 42 days of age prior to receiving the experimental diet. Liver fat of the SD strain rats was 33% as compared to 62% for the AES strain rats.

The incidence of renal hemorrhage and mortality was higher in the AES strain of rats than in the SD strain. When placed on experiment at 35 days of age, the AES strain rats had an incidence of 63% renal hemorrhage and 13% mortality. The incidence for SD strain rats was 12% and 0, respectively. Supplementation of the diet with 1% methanol was without apparent effect either for protecting against renal damage or for lipotropism.

Discussion. The experiments of this study

TABLE II. Effect of Age on Body Wt Gain, Kidney Damage, Mortality and Liver Fat of AES* and SD† Strain Rats‡ (8 Rats per Treatment).

Age at§ start of exp., days	Avg wt gain of survivors, g/2 wk		Incidence renal damage, %		Mortality, %		Avg liver fat (dry basis), %	
	AES	SD	AES	SD	AES	SD	AES	SD
21	16	39	100	100	38	38	55	—
28	35	54	100	63	38	0	67	65
35	53	61	63	12	13	0	64	66
42	58	69	0	0	0	0	62	33
35‖	56	70	50	50	25	0	64	65
42‖	—	66	—	0	—	0	—	26

* Alabama Experiment Station strain of rats (hooded).
† Sprague-Dawley strain of rats (albino).
‡ Basal diet supplemented with 150 μg vit. B_{12} and 10 mg folacin/kg.
§ The animals were maintained on an adequate stock diet from weaning (21 days) until the start of the experiment.
‖ 1% methanol added to the diets.

were conducted concurrently with those reported by Strength *et al.*(13) in which the AES strain of rats was used. These rats, when fed the basal diet supplemented with 0.04% choline chloride, developed renal damage, whereas only 63% of the SD strain rats had renal damage (Table II). In the absence of dietary vit. B_{12} and folacin, liver fat of the AES strain rats(13) was 12.3% as compared to 19.5% for SD strain rats (Table I), when the diet was supplemented with 0.20% choline chloride. At the 0.10% level of choline chloride plus vit. B_{12} and folacin, liver fat of the SD strain rats was 33.3% (Table I) as compared to 17.5% for the AES strain rats (13). At the 0.08% level of choline chloride plus vit. B_{12} and folacin, liver fat of the SD strain rats was 43.3% (Table I) as compared with 27.0% for the AES strain rats(13). These results indicate that, although weanling rats of the SD strain are apparently less susceptible to renal damage than AES strain rats, they have an initial higher choline requirement for maintenance of normal liver fat.

Supplementation of the diet with vit. B_{12} and folacin markedly suppressed the accumulation of fat in the livers of rats fed subprotective levels of choline. Under these conditions the minimum choline requirement for SD strain rats for lipotropism was 0.40% in the absence of vit. B_{12} and folacin and 0.20% in the presence of these factors. The concentration of choline in the liver parallels the removal of fat from the liver. Rats of the SD strain fed vit. B_{12} and folacin plus 0.15% choline chloride had liver choline values equal to those of rats fed 0.40% choline without vit. B_{12} and folacin.

Forty-two-day-old rats of either the AES or SD strain when fed the basal diet supplemented with vit. B_{12} and folacin grew rapidly and failed to develop renal hemorrhage even in the absence of a choline supplement. This indicates that animals of this age are capable of synthesizing sufficient choline and perhaps utilizing it more efficiently than weanling rats. The liver fat values indicate that 42-day-old SD strain rats are apparently able to synthesize and perhaps utilize choline more efficiently than AES strain rats when fed a diet supplemented with vit. B_{12} and folacin. This is a striking contrast with the results obtained with weanling rats of these strains.

Under the experimental conditions employed, the addition of methanol to the diet afforded no apparent protective action against renal hemorrhage.

Summary. (1) Weanling rats of the Sprague-Dawley (SD) strain have a lower choline requirement for protection against renal hemorrhage and a higher requirement for maintenance of normal liver fat than was observed in rats of the Alabama Experiment Station (AES) strain. (2) Forty-two-day-old rats of either strain grew rapidly and failed to develop hemorrhagic kidneys when they were fed the choline-deficient basal diet

13. Strength, D. R., Schaefer, A. E., and Salmon, W. D., *J. Nutrition*, in press.

supplemented with only vit. B_{12} and folacin. However, liver fat of the SD strain rats was 33% (dry basis) as compared to 62% for the AES strain rats. (3) The addition of vit. B_{12} and folacin to the basal diet supplemented with suboptimal choline chloride markedly suppressed the accumulation of fat in the liver, reduced the incidence of renal damage and increased the choline content of the livers of the SD strain of rats. Liver fat values averaging 12.8% (dry basis) were obtained with a 0.20% choline chloride supplement in the presence of vit. B_{12} and folacin as compared to a requirement of 0.40% choline chloride in the absence of vit. B_{12} and folacin.

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Reduction of X-ray Sensitivity of *Escherichia coli* B/r by Sulfhydryl Compounds, Alcohols, Glycols, and Sodium Hydrosulfite.* (18874)

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It has been shown(1) that oxygen has a marked effect on the survival of X-irradiated *Escherichia coli* B/r. A striking increase in survival was attained by saturating the bacterial suspensions with nitrogen before exposure to X-radiation. In this study, the X-ray sensitivity of *E. coli* B/r in the presence of 2,3 dimercaptopropanol (BAL), sodium hydrosulfite, and ethanol has been examined.

Suspensions of *E. coli* B/r cultured aerobically 20-24 hours in nutrient broth (Difco) were washed and resuspended in 1/15 M phosphate buffer. A 0.1 ml portion containing about 10^{10} cells was introduced into each of several 2 ml test tubes together with 0.9 ml of the test compound in phosphate buffer. Buffer solutions 0.04 M in sodium hydrosulfite and solutions 3.5 M in ethanol were used in examining the protective ability of these compounds. Dispersions equivalent to 0.04 M BAL were prepared by shaking in buffer a short time before addition to the suspensions. These were shown in other experiments(2) to be the maximally effective concentrations of these agents.

The diluted suspensions were cooled to 2°C and maintained at this temperature during the exposure to X-radiation. The source of X-rays was a Maxitron 250 unit operated at 250 kvp at 30 ma (3 mm added aluminum filter). The test tubes were held in a slanting position, at a 35° angle from the horizontal, under the X-ray tube by a circular lucite holder containing ice and ice water. The irradiated suspensions were diluted with nutrient broth and aliquots transferred to several broth agar plates, the average number of colonies developing in 24 hours at 37°C being used to calculate the number of surviving cells.

The log of the survival of *E. coli* B/r grown under aerobic conditions is a linear function of the X-ray dose, at least over the range 15,000 to 90,000 r. This relationship is illustrated in Fig. 1 for untreated suspensions and for suspensions which have been bubbled for 15 minutes with nitrogen immediately before the exposure period. As shown in Fig. 1-2, a similar relationship also obtains in the presence of BAL, sodium hydrosulfite, and ethanol.

The protective action of a chemical agent can be expressed simply as the ratio of the X-ray dose required to kill a given number of cells in the presence of the compound to that required in the absence of the compound. This method of presenting data is essentially that used by Kelner(3). "Isoefficiency ra-

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1. Hollaender, A., Stapleton, G. E., and Martin, F. L., *Nature*, 1951, v167, 103.

2. Burnett, W. T., Jr., Morse, M. L., Burke, A. W., and Hollaender, A.; Unpublished data.

3. Kelner, A., *J. Bact.*, 1949, v58, 511.