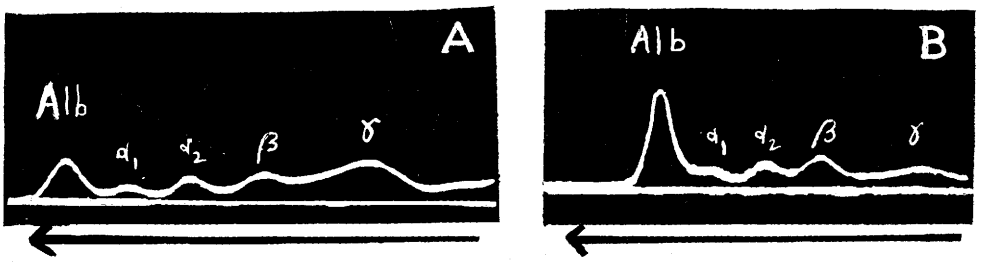

FIG. 2.
Electrophoretic diagrams of the serum proteins of one patient with *disseminated lupus erythematosus* before (A) and after therapy with cortisone and ACTH (B).

electrophoretic patterns obtained from the plasma of 2 cases of lupus erythematosus. Their data are somewhat difficult to interpret, since there seem to be some printing errors in their tables listing the protein composition of the plasma. Since phosphate buffer of pH 7.4 was used, they obtained no separation of the α_1-component from the albumin fraction. Due to turbidity they had difficulties in securing satisfactory diagrams.

In this study it was also found that occasionally the β- and γ-globulin boundaries were somewhat obscured, probably due to the presence of lipids or lipoproteins, even though the sera were not frankly lipemic. After high-speed centrifugation at low temperatures, a creamy layer rose to the top, leaving clear serum underneath, making it possible to obtain a well-differentiated electrophoretic diagram.

Summary. The electrophoretic distribution of the serum proteins of 20 patients with *disseminated lupus erythematosus* showed a lowered albumin content with a considerable increase in the α_2- as well as γ-globulin concentration. The sera of 5 patients were studied before therapy with cortisone and adrenocorticotropin (ACTH). After a clinical remission had been produced by these agents, it was found that the albumin and γ-globulin components progressed towards normal levels whereas the α_2-globulin fraction remained unchanged.

Thanks are due to Dr. George Baehr and the members of the First Medical Service for their cooperation throughout this study. The technical help of Sheldon S. Gilbert and Harold Corey is acknowledged with appreciation.

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Achromotrichia Due to Copper Deficiency. (17963)

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A purified diet, similar to diets used in many other laboratories has been employed in this laboratory as a presumably adequate diet for the albino rat. Recently it was observed that black rats on this diet developed an achromotrichia which could be prevented by liver or yeast. Further investigation disclosed that the activity of these substances was due to copper. Achromotrichia due to copper deficiency has been described previously(1-6). In most of these studies the ach-

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romotrichia developed on diets composed principally of milk or other crude dietary materials. The present report will describe a type of achromotrichia responding to copper which was produced with a highly purified ration which permitted normal growth and hemoglobin production.

Methods. Black, Long-Evans, male rats from the National Institutes of Health colony were housed individually in raised, wire bottom cages and placed on the diets when 21 to 29 days of age with weights of 25 to 59 g. Each group in individual experiments was balanced by litter and weight. The composition of the basal diet 1718B is indicated in Table I. The O&M salt mixture (7) and salt No. 550* were prepared in this laboratory following the original instructions. Copper analyses† were by the carbamate method(8). Hematocrits were determined as outlined by Van Allen(9). The degree of grayness was evaluated for each animal on an arbitrary 0 to 4 + scale. To facilitate the comparison of different groups of rats on the same or different diets the plus marks assigned to each rat in a group were totaled and divided by the number of rats in the group to give a Grayness Index. Thus, an index of 4 would represent maximum grayness in every rat. All of the Grayness Index values herein reported were calculated at the 8th experimental week.

TABLE I.
Composition of Diet 1718B.

	g
Casein (vit. free)	20.
Sucrose	71.7
Starch	1.
Wesson oil	3.
Salt O and M	4.
Cystine	.3
	mg
Thiamine	.2
Riboflavin	.3
Pyridoxine	.25
Ca pantothenate	2.
Choline chloride	100.
Inositol	10.
Nicotinic acid	2.
2-methylnaphthoquinone	.1
Biotin	.01
Folic acid	.1

Vit. A, D, and E as supplements.
A and D—2,000 and 400 U.S.P. units twice weekly.
E—3 mg tocopherol once each week.

Results. Development of gray hair. Rats maintained on the 1718B diet grew at a rate comparable to those on Purina Lab Chow, Table II, and seemed to develop normally except that within 5 to 6 weeks most of the animals developed marked graying of the hair. The first grayness was noted as early as 10 days and developed progressively reaching a maximum incidence and severity usually about 8 weeks after starting the diet. In about half of the rats the graying process appeared diffusely over the back. In others a

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* Salt No. 550 differed from O&M salts in that it had only 1/10 as much NaF and contained .25% added $CuSO_4 \cdot 5H_2O$.

† We wish to thank Mr. William C. Alford and his staff of the Microanalytical Laboratory of this Institute, who did all of the copper analyses reported in this paper.

‡ *Substances Active*—Whole Dried Liver 5%; Dried Brewers Yeast 5%; Dried Alfalfa 20%; $CuSO_4 \cdot 5H_2O$ 0.005%; Purina Lab Chow. *Substances slightly active*—Wheat germ 10%; O&M salts 8%; Salts No. 550, 4%. *Substances inactive*—Wheat 20%; Corn Meal 20%; Soybean Meal 10%; Fish Meal 10%; Whole dried milk 10%; Lactalbumin 10%; Casein 40%; Cerelose 72%; Starch 72%; Crisco 20%; Tyrosine, Phenylalanine and Tryptophan 0.2% each; Cystine 0.3%; Carrots and Kale fed separately ad lib; $CoSO_4 \cdot 7H_2O$ or $MnSO_4 \cdot 4H_2O$ 0.02% each; $FeSO_4 \cdot 7H_2O$ 0.18%; Liver extract 3 U.S.P. units or B_{12} 2 μg each given daily subcutaneously; Pantothenic acid 10, Niacin 10, Thiamin 1, Pyridoxine 1, Riboflavin 2, p-amino benzoic acid 100, Choline 200, Ascorbic acid 200 mg % each used separately; double quantities of vitamins used together as in 1718B diet.

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TABLE II.
Effect of Various Diets on Growth and Achromotrichia.

Diet	No. of rats	Cu in diet, μg	Gain in body wt, g/wk*	No. of gray rats†	Grayness index
Purina Lab Chow	10	21.5	21.4	0	0
1718B	80	14.6	20.0	68	1.7
" + 5% liver powder	30	20.0	24.7	5	0.3
" + 10% liver powder	10	25.6	27.0	0	0‡
" + 5% brewers yeast	20	18.3	22.6	0	0
" + 10% brewers yeast	10	22.0	24.4	0	0‡
" + 20% dried alfalfa	10	21.0	26.3	3	0.4
" + .02% $CuSO_4 \cdot 5H_2O$	30	64.6	21.2	1	0.03
" + 10% dried wheat germ	10	—	24.2	4	0.6
" + 4% O & M salt	10	15.5	21.4	8	1.2

* Growth rate calculated for first 4 wk on diet only.
† After 8 wk on the diet.
‡ Observed 12 wk.

TABLE III.
Amount of Copper Necessary to Prevent Achromotrichia When Added to 1718B Diet.

No. rats	Cu added to diet, μ/g	Growth, g/wk	No. showing detectable graying	Grayness index
9	.25	20.5	5	1.6
9	2.5	22.8	2	.3
10	12.5	22.9	0	.0
10	25.	22.	1	.2
9	50.	20.2	1	.1

definite pattern developed with a gray strip down the middle of the back, a patch at the base of the tail or a collar patch across the back of the neck. These patterns gradually disappeared as the remainder of the hair grayed so that the end result in every animal was a rather uniform coat of various degrees of grayness. Eighty rats on this diet showed a Grayness Index of 1.7. About 20% of these showed no graying, or graying too slight to assess accurately. A few of the rats developed hair which was a dull red rather than gray. Most of the rats with marked grayness showed considerable thinning of the hair with loss of much of the fine undercoat. Diet 1718B was completely ineffective within periods of 12 weeks in producing gray hair in adult rats which had been reared on stock rations.

Prevention of gray hair. A large number of substances were tested for their ability to prevent the achromotrichia‡ (Table II) by adding appropriate amounts to the 1718B diet at the expense of sucrose. Whole dried liver, 5%, or dried brewer's yeast, 5%, were quite effective. Large amounts of all the water soluble vitamins were without effect, singly or in combination. Pantothenic acid and p-aminobenzoic acid, which have been implicated in other types of achromotrichia, were completely inactive. When diet 1718B was supplemented with copper in amounts as small as 2.5 μg per g in the form of $CuSO_4 \cdot 5H_2O$, gray hair was largely prevented. Larger amounts were somewhat more effective (Table III).

Copper content of diets. It seems evident that 4% of O&M salt mixture supplied an insufficient amount of copper. Even when this salt mixture was increased to 8 percent, achromotrichia still developed (Table II). This mixture contained no copper added as such but depended on the other ingredients to supply trace metals as impurities. Analysis of this salt mixture showed 23 μg of copper per g which would supply less than 1 μg of copper per g to the whole 1718B diet. Analysis of the diet, however, showed 14.6 μg of copper per g indicating that other ingredients in diet 1718B supplied considerable

TABLE IV.
Effect of Ashing on Achromotrichia Preventing Effect of Liver Yeast and Alfalfa.

No. rats	Diet	No. of gray rats	Grayness index
10	1718B	8	2.2
8	" + liver ash	1	.12
10	" + alfalfa ash	3	.3
10	" + yeast ash	3	.6

Liver and yeast ash added to diet equivalent to 5% of starting material; alfalfa ash = 20% dried alfalfa.

TABLE V.
Copper Absorption of Rats on Various Diets.

No. rats	Diet	Total Cu intake, μg/rat	Total Cu in feces, μg/rat
5	1718B	398	19
5	" + liver	875	68
11	Gray rats on various diets	860	54.6
13	Non-gray rats on same diets as above	912	99

The intake and fecal excretion of Cu are presented as the totals for a 3-day period.

Both the gray and non-gray rats were on several diets, some of which usually did, and some of which usually did not prevent graying.

copper. The addition of 5% liver or yeast to Diet 1718B increased the copper content to 20 and 18.3 μg per g respectively, amounts sufficient to account for their achromotrichia preventing effect according to the amount of copper necessary when added to the diet as $CuSO_4 \cdot 5H_2O$ (Table III).

Effect of ashing. The fact that most, if not all, of the achromotrichia preventing effect of liver, yeast and alfalfa was due to their mineral content was confirmed by dry ashing these materials and adding the ash to the 1718B diet in amounts equivalent to effective concentrations of unashed material (Table IV).

Intestinal absorption of copper. It is to be noted that the addition of quite small amounts of copper, 2.5 μg per g, to the 1718B diet produced a striking reduction in the incidence of achromotrichia. This was true whether the copper was added as copper sulfate or as liver or yeast. The fact that such small amounts of copper produced such large changes even though the 1718B diet already contained more than 10 times this amount of copper suggested that some of the copper in the 1718B diet might be in a form unavailable for absorption. This was checked by determining the dietary intake and fecal excretion of copper. The results (Table V) make it evident that intestinal absorption of copper was quite efficient whether the rats were on achromotrichia producing or achromotrichia preventing diets. Since the feces are the principal route of copper excretion (10), it is probable that these rats were retaining most of the dietary copper. That efficiency in intestinal absorption of copper was not a determining factor in the development of achromotrichia was supported further by a comparison of the last two groups of rats in Table V. These rats were selected from a number of experiments using various diets some of which usually did and some of which usually did not prevent achromotrichia. It is clear that there was no significant difference in the efficiency with which these two groups retained copper.

Hematocrits. Hematocrit determinations on 30 rats after 8 weeks on the 1718B diet gave an average value of 46.3. Ten comparable rats on the copper supplemented 1718B diet showed an average hematocrit of 45.0. Other gray rats were observed for as long as 5 months without showing evidence of anemia.

Treatment Experiments. Rats with gray hair showed no tendency to revert spontaneously toward their normal black hair color when maintained for long periods on diet 1718B. Even shaving, to stimulate new hair growth, produced only more gray hair. In many animals the fur coat did not completely regenerate leaving bald areas after shaving. Gray rats began to grow black hair quickly if their diet was changed to Purina Lab Chow or if the diet 1718B was supplemented with 5% yeast or liver, 20% alfalfa or 0.02% $CuSO_4 \cdot 5H_2O$. Black hair was first noted about 10 days after the diets were changed

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and became progressively more pronounced and more complete within periods of 3 months. Purina Lab Chow produced complete return to a normal hair coat within this period. The 1718B supplemented diets produced complete regeneration in some animals but in others their effect was definite but incomplete. Usually it was the rats with far advanced grayness which failed to show complete regeneration of black hair. Rats with less severe achromotrichia often returned to normal within one month.

Discussion. It is clear that copper was the principal factor in the achromotrichia observed in these experiments. Copper is known to be an essential dietary element(11) and is essential for melanin formation since dopa-oxidase is a copper protein complex(12). Likewise, several reports have indicated that black hair contains more copper than light colored hair(13) although a few reports to the contrary have appeared(14). However, several considerations suggest that a factor or factors in addition to simple copper deficiency may have exerted some influence in the achromotrichia observed in these experiments. Rats subsisting on the Purina Lab Chow ration were never observed to develop graying. On the other hand, rats on the purified diets containing equivalent amounts of copper occasionally did develop some graying. Likewise in treatment experiments none of the copper supplemented purified diets were as effective in regenerating black hair as was the Purina Lab Chow. Occasionally rats on the 1718B diet supplemented with 5% liver developed mild grayness which would not respond to additional copper. It should also be noted that liver, yeast, alfalfa and wheat germ produced considerable growth stimulation. This effect was still greater after 8 weeks than at the 4-week period recorded in Table II. Copper, the ash from liver, yeast and alfalfa, the known vitamins, additional protein or fat, different carbohydrates and additional minerals were all ineffective in duplicating this growth effect.

It is probable that copper is required for normal hair growth in addition to melanin production. Gray rats in these experiments never developed spontaneous alopecia but often showed diffuse thinning of the hair with an unkept, dull appearance. If the hair was shaved, deficient hair regrowth leading to partial alopecia was observed. Copper seemed to stimulate both melanin production and hair growth when added to the diet of gray rats.

The quantitative requirement of the rat for copper under the conditions of our experiment can be calculated from the data in Tables II and III plus data on the daily food intake which were recorded for many animals. In terms of concentration in the diet, it is evident that 14.6 μg of copper per g of a diet supplying approximately 4 calories per g was adequate for normal growth and hemoglobin production, although approximately 20 μg of copper per g of diet was required to prevent achromotrichia. Based on the number of grams of food consumed per day, rats weighing an average of 150 g maintained body weight and hemoglobin but became gray on a daily copper intake of 0.143 mg and maintained their black hair color if the copper intake was 0.196 mg per day. Gortner(2) also noted that more copper was required to prevent achromotrichia than to prevent anemia. According to his data, 0.02 to 0.12 mg of copper per day was required, this apparently being in addition to an unspecified amount in the diet. Kiel and Nelson(1) found that 0.05 mg of copper per day would cure achromotrichia in rats on an iron supplemented whole milk diet which supplied 0.24 mg of copper per liter. Assuming that a 150 g rat would consume 50 ml of milk per day, achromotrichia was cured with 0.06 mg per day in their experiments, an amount considerably less than that necessary in our experiments. This is contrary to the results of Drabkin and Waggoner(15)

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who reported that low copper synthetic rations were more effective than whole milk diets in curing anemia. Others have disputed this finding however(11).

Conclusions. Black rats subsisting on a purified diet containing 14.6 μg of copper per g grew well and maintained normal hemoglobin levels but developed marked achromotrichia. The achromotrichia could be cured by adding copper sulfate to the diet and could be prevented by diets containing approximately 20 μg of copper per g. The activity of liver, yeast and alfalfa in preventing and curing the achromotrichia was due to their copper content. Evidence suggesting that the basal purified diet used in these experiments may have been deficient in an unidentified growth factor has been discussed.

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Formation of Neutralizing Antibody in Monkeys Injected with Poliomyelitis Virus and Adjuvants.* (17964)

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It has been long known that rhesus monkeys receiving repeated injections of poliomyelitis virus produce neutralizing antibodies. Recently Morgan, Howe, and Bodian(1) and Morgan(2) studied the effect of dosage, route of administration of the antigen and other factors on the hyperimmunization against this virus. No evidence was found that antibody formation was augmented by the incorporation of the Lansing type of virus into a water-in-oil emulsion with or without tubercle bacilli (1,3).

Our hope for the effectiveness of these adjuvants in relation to poliomyelitis virus was raised by results obtained recently with another neurotropic virus, namely, that of rabies(4). It was found that rabbits or guinea pigs, given a single injection of brain containing inactivated rabies virus emulsified in paraffin oil and one month later a few more injections of the same antigen in salt solution, produced neutralizing and complement fixing antibodies in great abundance.

Material and methods. As an immunizing agent, monkey spinal cord, containing active poliomyelitis virus of the Lansing type, was used. It was suspended in salt solution with the aid of a Waring blendor. The antigen was injected either in this form or emulsified in paraffin oil with or without the addition of a small amount of killed *Myco. butyricum.* The technic of emulsification and the ingredients used have been described previously (5). Immature rhesus monkeys (*Macacca mulatta)* as a rule, were given 5 simultaneous injections. The sites were the pectoralis, and the flexor muscles of the femur and the subcutaneous tissue of the nuchal region. One monkey (Group IIIa) received the primary injection in the footpads. The "booster" injections were the same for all groups, namely, the antigen in salt solution. The data are given in Table I.

The neutralizing antibodies were titrated using 4 or 5 fold serial dilutions of serum

* Aided in part by a grant from The National Foundation for Infantile Paralysis, Inc.

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