TABLE III. Potentiation of Sodium Hexobarbital by Win 13,645-5 Administered Intrav. (10 Mice/Dose).

Win 13,645-5 dose, mg/kg intrav.	Sodium hexobarbital		No. of mice losing righting reflex	$ED_{50} \pm$ S.E., mg/kg
	Dose, mg/kg	Route		
.32	40	Intraper.	1	.56 ± .10
.50	"	"	4	
.80	"	"	8	
.10	25	Intrav.	8	
.20	"	"	9	
Saline	"	"	0	

minutes, before there could be any fall in body temperature. A control group given saline instead of Win 13,645-5 showed no potentiation of the i.v. subhypnotic dose of sodium hexobarbital.

Discussion. It is often difficult to separate 2 properties possessed by a drug unless they are produced at different dose levels. In the case of Win 13,645-5, reduction in body temperature of mice and potentiation of the sedative action of sodium hexobarbital both occur at the same dose levels. The fact that this is so does not necessarily mean that one property is the cause of the other. In the procedure used by others where large anesthetic doses of the barbiturate are given, and potentiation determined by measuring prolongation of anesthesia it is quite possible that the concurrent hypothermia will retard metabolism of the barbiturate. The fact that potentiation of sodium hexobarbital can be demonstrated within 2 minutes of intravenous administration of Win 13,645-5 indicates that this potentiation is independent of hypothermia.

Summary. A modified test for measurement of the property of a phenothiazine derivative to potentiate the action of barbiturates has been described. Using this technic, it has been shown that although there is some correlation between degree of hypothermia produced and potency of such a derivative to potentiate barbiturate sedation, these pharmacologic properties are primarily independent of each other.

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Pre-staining Procedure for Electrophoretic Study of Serum Lipoproteins.* (24161)

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McDonald(1) introduced a new staining procedure for serum lipoproteins which involved staining of lipids before applying serum to paper for electrophoretic separation. This procedure as modified in our laboratory largely overcomes some of the difficulties encountered in previous studies of electrophoretic separation of α and β lipoproteins utilizing post-staining procedures. Advan-

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tages of the pre-staining procedure include: conservation of time and reagents by omitting staining and rinsing of paper strips, better contrast between stained lipoprotein bands and unstained paper background, and also elimination of objection that rinsing solutions capable of washing dye from paper background may extract dye from lipoprotein bands as well. This paper presents a detailed study comparing the modified pre-staining procedure with the post-staining procedure for separation of serum lipoproteins by paper electrophoresis. It demonstrates advantages of the pre-staining procedure, and also presents clinical application of this procedure in a study of lipoprotein patterns in persons of differing age and sex.

Method. Acetylated Sudan black B was prepared as described by Lillie and Burtner (2). Occasionally a preparation failed to stain the lipids with any greater intensity than the nonacetylated product. When this occurred, the dye was discarded and a new batch prepared. Successful preparations of acetylated Sudan black B prepared from the same or different lots of Sudan black B showed no difference in lipid-staining properties. Five to 10 ml blood samples, taken in the post-absorptive state, were drawn into a clean, dry syringe; the needle was removed to prevent hemolysis and the sample carefully transferred to a glass test tube. After the clot was well formed, the sample was centrifuged in a refrigerated centrifuge at 5°C for 5 minutes at 2000 rpm. The serum was transferred to another tube and a saturated solution of acetylated Sudan black B (excess added to 100% alcohol, heated to 70°C for 10-15 minutes and filtered) was added with stirring, maintaining a serum-to-dye ratio of 10:1. After an hour the stained sample was centrifuged to remove excess dye particles. Twenty-five microliters of the stained serum were applied by means of a Spinco stripper to Whatman 3 MM filter paper strips on a Spinco Durrum-type electrophoresis cell containing veronal buffer (pH 8.6, 0.075 ionic strength) and a potential of 200 v was applied for 75 minutes. The strips were dried and scanned by means of a Spinco Analytrol. The results were expressed as β/α ratio, obtained by dividing the area under the β-lipoprotein curve by the area under the α-lipoprotein curve. Since changes in the β and α-lipoprotein values usually appear to be reciprocal, this type of measure is more sensitive than percentage values. To compare pre- and post-staining technics, one serum sample was divided into 24 aliquots. Twelve of these aliquots were electrophoresed and stained according to the method of Swahn (3). The remaining 12 were prestained and electrophoresed according to the method here described. To test the reproducibility of this pre-staining procedure and the subsequent electrophoretic separation of lipoproteins, 6 serum samples were studied. Each was divided into 9 aliquots. After staining each aliquot, 8 strips were prepared for electrophoresis from 1 aliquot and electrophoresed on separate runs. One strip was prepared from each of the remaining 8 aliquots and electrophoresed in the same cell. In a study of the lipoprotein values with respect to age and sex, the lipoprotein patterns of 4 groups of subjects were determined over a 4-week period. The 4 groups included: young men, young women, older men, and older women. The young men were medical students; the young women, nursing students; and the older men and women were selected from geriatric wards of the San Bernardino County Hospital. All of the participating subjects maintained their usual pattern of living without major changes in activity or diet during the course of the experiment.

Results. An example of typical strips obtained from pre- and post-staining procedures is shown in Fig. 1. It will be seen that the patterns are similar, but the lipoprotein bands from the pre-stained serum stand out more distinctly and the densitometer curve is sharper. Data in Table I indicate that reproducibility of the pre-staining procedure is better than with our former practice of staining lipoproteins after electrophoretic separation on paper.

Comparison of β/α lipoprotein ratios of 8 strips from one pre-stained aliquot with ratios of the 8 strips from individually prestained aliquots showed no significant dif-

TABLE I. Lipoprotein Ratio as Determined by Pre-staining and Post-staining a Single Serum Sample. 12 strips/series.

	Mean	S.E.	"T"	"P"
Pre-staining	3.97	.07		
Post-staining	4.51	.26	2.01	0.1

ference (Table II). This indicates that the procedure is reproducible both in electrophoretic separation and in staining of lipoproteins.

The mean β/α lipoprotein ratio for the group of young women was significantly lower than the ratios for each of the other 3 groups ($P<0.001$). Older women had a significantly lower ratio than older men ($P<0.05$). Young men also had a somewhat lower ratio than older men ($P<0.1$). A summary of these data is shown in Table III. These results indicate differences between the groups as measurements of cholesterol and lipoproteins by other investigators using different technics have shown(4-9).

TABLE II. Summary of Reproducibility Study of Pre-staining Procedure for Serum Lipoproteins.

Sample	Mean values, β/α ratio	S.D.	S.E.	t
1. a	6.21	.53	.04	1.64
b	6.61	.43	.16	
2. a	2.77	.19	.07	.69
b	2.69	.26	.10	
3. a	2.71	.23	.08	.82
b	2.87	.50	.18	
4. a	2.50	.24	.09	.73
b	2.42	.20	.07	
5. a	2.14	.16	.06	.80
b	2.13	.32	.11	
6. a	2.30	.15	.05	.98
b	2.38	.22	.08	

a—8 strips, all from one pre-stained aliquot.
b—8 strips, one from each of 8 individually pre-stained aliquots.

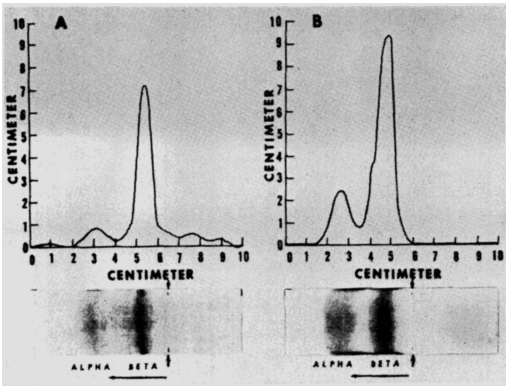

FIG. 1. Typical paper electrophoretic patterns and electrophoretograms of serum lipoproteins post-stained (A) with Sudan black B and pre-stained (B) with acetylated Sudan black B. Small arrows indicate point of serum application.

An expected difference in lipoprotein values is evident within the different groups and the values of individual subjects varied some from week to week. Such changes should be kept in mind where one attempts to relate a pathological condition to minor changes in the lipoprotein pattern.

In attempting to account for some of the changes seen in β/α lipoprotein ratios, it was found that the elevated values in the medical student group during the second and third weeks of observation coincided with a series of examinations. The possibility of a relationship between stress and lipid levels has been investigated further and some preliminary data have been reported(10).

Summary. A technic for staining serum lipoproteins prior to electrophoretic separation is reported. This procedure results in better reproducibility than is derived in the usual practice of staining paper strips after electrophoresis and also conserves on time and cost. When serial determinations are made with this procedure, in persons of differing age and sex, the lipoprotein ratios show differences be-

TABLE III. Summary of Differences in the β/α Lipoprotein Grouped According to Age and Sex.

Subjects	Mean age	Wk 1	Wk 2	Wk 3	Wk 4	4 wk avg	Area avg‡ α	Area avg‡ β
Young women (16)*	20	1.68 ± .11†	2.29 ± .12	1.93 ± .93	1.95 ± .12	1.96 ± .11	30.1	55.9
Older women (8)	84	2.87 ± .32	3.38 ± .21	2.83 ± .09	3.56 ± .29	3.16 ± .23	23.5	65.8
Young men (19)	25	2.94 ± .18	3.77 ± .16	3.48 ± .17	3.05 ± .24	3.31 ± .19	17.6	51.5
Older men (12)	74	3.44 ± .21	3.33 ± .22	4.54 ± .26	4.01 ± .20	3.83 ± .22	23.8	81.9

* No. of subjects. † Mean ± S.E. ‡ Avg area under peaks of α and β lipoproteins (mm^2).

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Temperature-Sensitive Requirement for Methionine or p-Aminobenzoic Acid in Strain of *Escherichia coli*.* (24162)

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It has been known for at least 50 years(1) that naturally occurring strains of *Escherichia coli* can be cultured readily in simple synthetic media lacking specific growth factors. A simple source of carbon and energy such as glucose, glycerol or lactic acid and inorganic ammonium salts as sole source of nitrogen suffice for growth of the majority of *E. coli* strains. Occasional strains have been isolated, unable to grow in a minimal salts-glucose medium(2). This report deals with a naturally occurring strain of *E. coli* which behaves like the species prototype when grown at room temperature (21-24°C), but will not grow at 37°C unless the medium is supplemented with either methionine or p-aminobenzoic acid (PABA). This anomalous behavior is apparently associated with an abnormal reaction whereby homocysteine interferes with the role of PABA in synthesis of methionine.

Materials and methods. Escherichia coli, strain WM-13, was one of several strains isolated from clinical material at the Hospital for Women's Medical College of Pennsylvania and obtained through the courtesy of the late Dr. L. J. Kimmelman. It was identified as *E. coli* on the basis of morphology, lactose fermentation and typical IMViC reactions. It is susceptible to the action of coliphages T1, T3, T6 and T7, but resistant to T2, T4 and T5. It serves as sensitive indicator strain for temperate phages *lambda* and *w2*(3), and can be lysogenized by these phages. Growth requirement is unchanged by lysogenization. When lysogenized with *lambda* phage it loses its sensitivity to lysis by T1 and T7 and production of mature *lambda* phage can be induced with ultraviolet irradiation or with L-azaserine. Growth analyses were carried out in the salts-glucose (S-G) medium of Gots and Chu(4). The medium was inoculated to final concentration of 10^5 cells/ml with a bacterial suspension harvested from overnight culture in nutrient broth and washed twice by centrifugation with minimal S-G media. Growth was recorded by turbidimetric readings with a Klett-Summerson photoelectric colorimeter equipped with green filter (#54).

Results. When incubation was carried out at 37°C, strain WM-13 was unable to grow in unsupplemented S-G medium. When medium was supplemented with either casein hydrolysate (0.1%) or with a mixture of B vitamins, even greater growth was obtained than in nutrient broth. Addition of single amino

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