FIG. 4. Atherosclerotic plaque—human abdominal aorta. Bellevue Hospital Autopsy #39551. Alizarin. × 56.
FIG. 5. Calcification of aortic valve. Same case as in Fig. 4. Alizarin. × 128.
FIG. 6. Calcification of myocardial fibres. Same case as in Fig. 4. Alizarin. × 128.

uranium damaged kidneys, the method revealed minute foci of accumulation from 12-48 hours before an increase could be unequivocally demonstrated by quantitative microanalysis. The correlation of the histochemical test with quantitative analysis of the tissue was investigated in a series of sections that had been taken from the kidneys of rats after injury with uranium. On the basis of the alizarin stain 8 kidneys were ranked from greatest to least calcification. The sequence thus obtained correlated closely with the sequence based upon quantitative analysis (Table III).

TABLE III. Comparison of Tissues According to Amounts of Calcium Present.

Quantitative analysis mg Ca / g tissue	Rank	Alizarin stain Rank
8.82	1	1
6.48	2	3
5.06	3	2
2.87	4	4
2.60	5	6
1.78	6	5
1.27	7	8
1.17	8	7

The kidneys from 8 uranium-damaged rats(7) were subjected to microanalysis for calcium. Amounts of calcium found are shown at left. Sections of kidneys from each of these animals were stained with alizarin red S, and the amounts of calcium shown by this stain were ranked from greatest to least. The column on the right indicates this ranking, which is to be compared with the absolute rank, shown in the middle column.

Summary. A technic is described for histochemical definition of calcium. It is specific for calcium in the presence of magnesium, approximately 50 times as sensitive as previous methods based upon the use of alizarin, and capable of localization to within a few micra.

It is a pleasure to acknowledge the aid of Dr. Vincent P. Dole throughout the course of this work.

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Linkage Studies of Genes for Sickling and MNS Blood Groups: Negative Findings. (19662)

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Snyder, Russell and Graham(1) presented evidence of linkage between the genes for MN blood types and the gene for sickling of erythrocytes. Subsequent studies by Snyder, Clark and Moore(2), and Snyder(3) gave additional supporting evidence for linkage between these two genes. The above studies were based on a total of 85 families, 12 of which were usable for linkage studies. Race *et al.*(4), in studies on the MN and S groups in 123 English families observed close linkage between the genes for these two blood groups, no recombination being observed in 82 children capable of disclosing it. The

‡ Deceased July 14, 1952.

prediction of the allele s by Sanger and Race (5) and the subsequent discovery of an anti-s serum by Levine *et al.*(6), further supported the concept of linkage between S and MN. While the present investigation was under way, Neel and Hanig(7) in their study on the inheritance of the MN and S factors in American Negroes, found no evidence of recombination among 18 children of 5 matings, thus lending confirmation to the observations of Race *et al.*, in a different population group.

The present investigation was undertaken with the hope of confirming the above studies and determining the relative position of the genes for MN, S and sickling.

Procedure. This study is based on an analysis of 325 individuals from 55 Negro families of which 52 are complete, each consisting of father, mother, and one or more children. In addition to the determinations for MN, S and sickling, tests for the Rh (D,C,E) factors and the ABO blood groups were also performed in order to exclude as many illegitimate children as possible. Only about one-third of the families lent themselves to the study of linkage between MN, S and sickling. The data were subjected to analysis by Finney's method for the detection of linkage(8). Several of Finney's mating types were used and all gave comparable results. Only the following mating types will be reported here:

Finney Type				
20	MM or NN	si si*	×	MN Si si
21	MN	si si	×	MN Si si
22	MN	Si si	×	MN Si si

For the 17 families analyzed for linkage between MN and sickling the total score, $\Sigma(\lambda)$, was found to be -2. The variance of the score, $\Sigma(\kappa)$ was found to be 51. Because $\Sigma(\lambda) = -2$, is less than $1.64\sqrt{\Sigma(\kappa)} = 11.71$, no linkage could be demonstrated.

Although the quantity of information available for analysis of linkage between S and sickling, and MN and S is small, it will be included here for the sake of completeness. For the 5 families analyzed for linkage between S and sickling, the $\Sigma(\lambda)$ amounted to $+3$ and the $\Sigma(\kappa)$ to 13. The $\Sigma(\lambda)$ was calculated to be less than $1.64\sqrt{\Sigma(\kappa)}$ and consequently again no linkage could be shown. For the 5 families analyzed for linkage between MN and S, $\Sigma(\lambda)$ was $+9$ and $\Sigma(\kappa)$ 19. Therefore, because $1.64\sqrt{\Sigma(\kappa)}$ is exceeded by $\Sigma(\lambda)$, linkage is indicated.

The entire family material was submitted to Drs. R. R. Race and Ruth Sanger† who very kindly analyzed the data according to the u-statistics of Fisher(9). The families were scored by them in two ways: 1) assuming that sickle cell anemia is the homozygous condition and the sickle cell trait the heterozygous (10). 2) not making the above assumption and judging heterozygotes as those persons with one or more negative children. When the mating was sickler x sickler, only the negative children were scored. Thus 23 families were analyzed for linkage between the MNS system as a whole and sickling, using evidence from both homozygotes and heterozygotes(1). This study yielded values of $\Sigma(\lambda) = +9$ and $\Sigma(\kappa) = 117$. As the $\Sigma(\lambda) = +9$ does not exceed $1.64\sqrt{117} = 17.74$, linkage could not be demonstrated. Without making a distinction between the heterozygote and homozygote(2), 17 families were recalculated for linkage with MNS and values of $\Sigma(\lambda) = +4$, and $\Sigma(\kappa) = 106$ were found. Because $\Sigma(\lambda) = +4$ is less than $1.64\sqrt{106} = 16.89$, no linkage was indicated.

All pedigrees, a frequency study of MNS and sickling on unrelated Negroes, as well as methods for the detection of sickling and details of crossover analysis will be reported elsewhere.

Summary. Analysis of data supplied by 55 Negro families comprising 325 kindred, failed to reveal linkage between the MNS blood group genes and the gene for sickling. These findings are not in accord with those of Snyder and coworkers. Thus an example of autosomal linkage, for which Snyder *et al.* presented evidence, is now in doubt and warrants further investigation. Data ascertainable for linkage calculations between the blood group S and sickling, and MN and S were very small.

* non-sickler

† Lister Institute, London.

Linkage between S and sickling could not be found. The blood groups MN and S afforded evidence for linkage thus fitting into the very considerable evidence on this point for Caucasians.

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Effect of Dihydroergotamine on Epinephrine Response in the Hind-Limb of Dogs.* (19663)

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The vasoconstrictor action of exogenous epinephrine can be blocked readily with the various ergot alkaloids(1). Conclusions based primarily on changes in the systemic blood pressure indicate that these alkaloids will not block the vasodilator response of epinephrine. On the other hand the ergot alkaloids will block the inhibitory action of epinephrine on isolated intestinal strips(2-4). There may be no similarity between the mechanism of inhibition of the intestine by epinephrine and the vasodilator response on the vascular system. Nickerson(1) objects to the use of the intestine to investigate the effect of ergot compounds on the inhibitory action of epinephrine because of the presence of intramuscular parasympathetic ganglia in this tissue and the potentiation of the cholinergic response with some of the ergot alkaloids. The experiments reported here were designed to determine the influence of ergot alkaloids on the response of the vascular system of the hind-limb of dogs to intra-arterially injected epinephrine.

Method and material. Dogs of either sex and weighing between 12 and 23 kg were anesthetized with 25 mg/ kg of thiopental and 40 mg/kg of chloralose. Through a mid-line abdominal incision, all branches of the lower 5 to 8 cm of the abdominal aorta and inferior vena cava, except the common iliac artery and vein for the experimental leg, were ligated. Blood flow from the experimental hind-leg was measured with a bubble flowmeter inserted into the common iliac vein. The blood was returned to the inferior vena cava by way of the opposite common iliac vein. The temperature in the flowmeter was maintained at 37°C by means of a water jacket. Blood pressure was recorded from the common iliac artery of the opposite leg and the heart rate was recorded with the electrocardiograph. The intra-arterial injections were made through a cannula inserted into the middle sacral artery. Intravenous heparin was given periodically throughout the experiment. Fresh solutions of synthetic l-epinephrine,† dihydroergotamine methanesulfonate and Hydregine (CCK-179) were prepared in a 0.9% sodium chloride solution. All intra-arterial injections were of a 2 ml volume and were given over a one minute period.

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