cell is not penetrated by the antihistaminic drug, requires that the site of action of histamine be at a surface to which *exogenous* histamine is carried, by the circulation, by penetration through cells, or by passage between cells. In this circumstance it is conceivable that *endogenous* histamine may, by arising within the cell, reach and act upon the surface in a manner not open to inhibition by the agent used. This is unlikely. "Benadryl" is a small, ionizable molecule which readily passes from the circulating blood to the extracellular spaces. There is no evidence to indicate that it is unable to traverse the cellular structure of the vessel wall in its passage. Clinical studies[13] have shown that those allergic manifestations held to be due to an increase in *endogenous* histamine activity are reduced by antihistaminic drugs. This requires that the drug reach the site of action of the *endogenous* histamine liberated in these disorders.

The term "antihistaminic" indicates that these drugs possess the ability to inhibit the biological effects of histamine. It would be expected that a "histamine-like" substance "with the biological properties of histamine" would be similarly inhibited. This cannot be determined, of course, until such substance is actually demonstrated.

Thus the failure of antihistaminic drugs to reduce the vasodilatation of reactive hyperemia in man can be considered to mitigate strongly against the generally accepted concept that histamine or a histamine-like substance is the primary factor in the vasodilatation of reactive hyperemia.

The unmodified persistence of the capacity for reactive hyperemia after sympathetic denervation indicates, as Lewis pointed out,[3] that this phenomenon is not dependent upon sympathetic integrity. The antihistaminic drug failed to modify reactive hyperemia in the sympathectomized limb. This demonstrates that the drug does not normally tend to evoke a compensatory reduction in sympathetic activity which could mask an effect on reactive hyperemia.

Summary and Conclusions. 1. In 6 subjects reactive hyperemia was produced in the legs by release of arterial occlusion maintained for from one-half to 10 minutes. Blood flow in the foot and distal portion of the leg was measured with a venous occlusion plethysmograph. The initial inflow rate immediately following release of arterial occlusion is taken as representative of the degree of vasodilatation resulting from the occlusion.

2. After administration of effective amounts of antihistaminic drugs the reactive hyperemia was not diminished.

3. The mechanism of reactive hyperemia has not been shown to be due to release of histamine or "H-substance." It is as yet unexplained.

[13] Feinberg, S. M., *J. A. M. A.*, 1946, **132**, 702.

16782 P

Nucleic Acid Content of Chromosomes of Normal and Leukemic Mouse Spleen.*

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It has recently been shown by Mirsky and Ris[1] that chromosomes may be isolated from the resting nucleus. These isolated chromosomes are made up chiefly of desoxypentose nucleic acid (DNA), histone, and the

* The expenses of this investigation were defrayed by grants from the Office of Naval Research, the National Cancer Institute of the United States Public Health Service, the Finney-Howell Foundation, and the James Foundation of New York, Inc.

[1] Mirsky, A. E., and Ris, H., *J. Gen. Physiol.*, 1947, **31**, 1, 7.

TABLE I.
The Nucleic Acid and Nitrogen Content of the Whole (W) and Residual (R) Chromosome Fractions of Normal and Leukemic Mouse Spleen.

	Exp. No.		Nitrogen—R/W, %	DNA, γ/γ N	PNA, γ/γ N
Normal	1	W		2.45	
		R	14	0.15	0.18
	2	R	—	0.13	0.19
	3	W		2.08	
		R	18	0.05	0.19
Leukemic	1	R	14	0.17	0.32
	3	W		2.77	
		R	23	1.21	0.36
	4	W		2.52	
		R	15	0.19	0.45

"residual chromosomes," which contain protein, pentose nucleic acid (PNA), and possibly a small amount of DNA. In this laboratory the whole and residual chromosomes of normal and leukemic mouse spleen have been isolated by the method of Mirsky and Ris.[1] The preparations have been examined microscopically and analyzed chemically for their DNA, PNA, and nitrogen contents.

Materials and methods. Mice of the Akm strain, about 3 months of age, were used. When injected with leukemic spleen minced in saline (strain 9421) they developed advanced leukemia in 8 or 9 days. The mice were sacrificed by spinal fracture, and the spleens removed immediately. The spleens of the normal controls averaged 180 mg in weight, while the leukemic spleens averaged 600 mg in weight. About 9 g of spleen were homogenized in 0.88 M sucrose, for cell fractionation studies which will be described elsewhere.[2] The nuclear fraction was resuspended in 0.15 *M* NaCl. The nuclei were broken in a refrigerated Waring mixer and the chromosomes isolated.[1] The progress of the isolation was followed microscopically by smears stained with Wright's stain.

Samples of the whole chromosomes were fixed and stained with Feulgen-light green and by other methods, and examined microscopically for traces of cytoplasm.† The nucleohistone was removed by extraction with *M* NaCl until the washings showed practically no absorption of light at 260 mμ. The residual chromosomes were suspended in *M* NaCl. Nitrogen was determined by Nesslerization.[3] The nucleic acids were extracted with hot trichloroacetic acid and analyzed for DNA by the diphenylamine test and for PNA by the orcinol test.[4]

Results. The whole chromosome fraction was fairly free of light green-staining material on microscopic examination. The residual chromosomes were badly clumped and could not be examined in detail.

Chemical analysis (Table I) showed that the DNA content of the leukemic chromosomes was the same as or slightly higher than that of the normal chromosomes. Both sets of values correspond to a DNA content of about 37% of the total chromosome, which is similar to the values found for "chromatin threads" from leukemic mouse spleen and rat leukemic tumors[5] and for whole chromosomes from calf thymus.[1]

The PNA content of the whole chromosome fraction, as determined by the orcinol reaction in the presence of a large excess of DNA, is only an approximate value. Only traces were found in the normal chromosome fraction, and slightly more (0.1 γ per γ of N) in that from leukemic spleen.

The residual chromosome fraction from

[2] Petermann, M. L., Alfin-Slater, R. B., and Larack, A. M., *Cancer*, in press.

† Through the courtesy of Dr. John J. Biesele.

[3] Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric technics and related methods for the study of tissue metabolism, Minneapolis, 1945.

[4] Schneider, W. C., *J. Biol. Chem.*, 1945, **161**, 293; 1946, **164**, 747.

[5] Claude, A., and Potter, J. S., *J. Exp. Med.*, 1943, **77**, 345.

both normal and leukemic spleens contained about 17% of the nitrogen of the whole chromosome fraction (Table I). The PNA contents, on the other hand, were strikingly different. Whereas the residual chromosome fraction from normal mouse spleen contained only 0.18-0.19 γ of PNA per γ of nitrogen, that from leukemic spleen contained 0.32 to 0.45 γ of PNA per γ of nitrogen, an increase of nearly 100%.

Discussion. It is of great importance to determine whether the excess PNA found in the residual chromosome fraction of leukemic spleen is an integral part of the chromosome structure or is contained in some other constituent of the preparation. The cytoplasm of the leukemic spleen cells contained large amounts of PNA.[2] That any significant amount of cytoplasm was left in these preparations seems unlikely, however, since careful microscopic observation failed to reveal it. The whole "nuclear fraction" of the leukemic spleens, however, was also extremely rich in PNA.[2] This is in agreement with the findings of Thorell,[6] who has shown, by ultraviolet spectrophotography, that the nucleus of the leukemic white cell contains 2 to 4 nucleoli rich in PNA, which are absent from the normal mature white cell. Since nucleoli sometimes remain attached to isolated chromosomes,[1] it is probable that some of the excess PNA found in the leukemic residual chromosome preparations is located in associated nucleoli; how much cannot be decided until preparations are obtained which do not clump so badly, and can be examined microscopically.

Summary. The residual chromosome fraction isolated from leukemic mouse spleen contained from 0.32 to 0.45 γ of pentose nucleic acid per γ of nitrogen, while that from normal spleen contained 0.18-0.19 γ per γ of nitrogen.

[6] Thorell, B., Studies on the formation of cellular substances during blood cell production. London, 1947.

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Isolation of a Streptomycin-Resistant Organism Capable of Utilizing Streptomycin for Growth.

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Since the introduction of streptomycin in 1944, the appearance of streptomycin-resistant variants in otherwise sensitive strains of microorganisms has been observed repeatedly. Miller and Bohnhoff[1] reported the isolation of variants of meningococci not only resistant to streptomycin but capable of growing only in its presence. These organisms were dependent upon streptomycin for multiplication both *in vitro* and *in vivo*. Paine and Finland,[2] described dependent variants of other bacterial species including *Staphylococcus aureus, E. coli, Ps. aeruginosa,* and *P. morgani.* More recently, 13 streptomycin-dependent strains of *M. tuberculosis* have been isolated in our own laboratory[3] from mice infected with the $H_{37}Rv$ strain and subsequently treated with streptomycin for a period of 31 days. The possibility that these organisms are capable of utilizing the nitrogen of streptomycin for growth has been suggested.

In the present communication, the isolation of a streptomycin-resistant organism capable of growing both in streptomycin as the sole source of nutrient and in broth containing no streptomycin is discussed.

[1] Miller, C. P., and Bohnhoff, M., *Science,* 1947, **105**, 620.

[2] Paine, T. F., and Finland, M., *Science,* 1948, **107**, 143.

[3] Lenert, T. F., and Hobby, G. L., *Am. Rev. Tuberculosis,* 1949, in press.