TABLE IV.
Effect of Salivary Mucin on Infection with LEE and PR8 Viruses.

50% E.I.D.* of virus	Mucin mg	Time mucin given in relation to virus inoculation	No. eggs	RBC agglutination 48 hrs after inoculation
100 LEE	5	1 hr before	5	5/5
100 "	—	—	6	6/6
10 PR8	25	1 " "	5	4/5
10 "	—	—	6	6/6

* E.I.D.—Egg infective dose.

gested that influenza virus has a direct enzymic action on mucin in the form of purified blood group substances. Burnet[5] thinks that the chief significance of this finding is that it provides a partial basis for the specific localization of infection by viruses of the mumps-influenza group to glands and mucous surfaces. Our failure to secure any evidence of viral action on human salivary mucin and hog gastric mucin indicates that the chemical composition and/or the molecular configurations of mucins derived from different sources are not identical. Supporting evidence for this viewpoint is contained in a recent report which shows that there are wide variations in the physical characteristics including the isoelectric point of mucin obtained from various levels of the gastro-intestinal tract of the hog.[6] The alteration of the mucinous blood group substances does not result in any alteration of the serological specificity of mucin[2] which again suggests that the change might involve only a slight molecular rearrangement.

Summary. Human salivary mucin and hog gastric mucin do not inhibit the hemagglutination induced by the PR8, LEE, and Swine strains of influenza virus and these mucins do not inhibit the multiplication of PR8 or LEE in the developing chick embryo. There was no turbidimetric or viscosimetric evidence of viral action on these mucins. Influenza virus was also unable to modify the conductivity of the human salivary mucin or the hog gastric mucin.

[5] Burnet, F. M., *Austral. J. Science*, 1947, **10**, 21.

[6] Domini, G., *Arch. fisiol.*, 1941, **41**, 36.

16349

Effects of Ether and Nembutal Anesthesia upon Blood Concentration of the Rat.

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The effects of ether and nembutal anesthesia on the blood concentration have been reported by several investigators. Anesthesia by nembutal results in hemodilution in cats[1] and in dogs.[2,3] Anesthesia by ether has been found to produce hemoconcentration in dogs[4-7] and

[1] Hamlin, H., Essex, H. E., and Mann, F. C., *Am. J. Physiol.*, 1939, **125**, 713.

[2] Hausner, E., Essex, H. E., and Mann, F. C., *Am. J. Physiol.*, 1938, **121**, 387.

[3] Hahn, P. F., Bale, W. F., and Bonner, J. F., Jr., *Am. J. Physiol.*, 1943, **138**, 415.

[4] Barbour, H. G., and Bourns, W., *Am. J. Physiol.*, 1924, **67**, 399.

[5] McAllister, F. F., *Am. J. Physiol.*, 1937, **119**, 363; 1938, **124**, 391.

[6] Bollman, J. L., Svirbely, J. L., and Mann, F. C., *Surgery*, 1938, **4**, 881.

[7] Searls, P. W., *J. A. M. A.*, 1939, **113**, 906.

a similar but less marked effect is believed to appear in man.[8,9] However, in etherized cats[10] and rabbits[11] no such blood concentration occurs.

It is clear that a species difference exists in the response of the above animals to ether, and with further investigation a difference may appear among other experimental animals. In this respect the laboratory rat has been strangely neglected; no study of the effect of ether or nembutal on the blood concentration of the rat has been reported in the literature.

The problem introduced is important on two counts: (1) the correct interpretation of blood data obtained from anesthetized animals requires a knowledge of the changes produced by the anesthesia employed; (2) there is danger in applying information gained from one species to experiments involving another.

Methods. Male albino rats, weighing about 200 g, were studied in 2 groups of 20 rats each, for the effect of anesthesia on the blood concentration. The effects of ether and nembutal were determined on separate groups. Ether was administered by the respiratory route until the animal was prostrate and all reflexes absent. Nembutal was given by intraperitoneal injection in the amount of 0.2 ml of a 10% solution. The rats were placed in a restraining box and blood samples were taken before and immediately after anesthesia by clipping the tail and using the free-flowing blood. Additional samples were obtained following anesthesia by the use of cardiac puncture. Determinations were made on the red cell count and on the specific gravity of the blood. The cell count was determined by employing standard clinical dilution pipettes, diluting fluids, and hemocytometers. The specific gravity was determined by the copper sulfate method of Phillips *et al.*[12]

Results. It is clear from the averages in Tables I and II that both ether and nembutal produced a hemodilution as expressed in the reduced red cell numbers and specific gravity. These differences have been shown to be significant by application of the Fishers' t test. The greater dilution of the blood drawn from the heart as compared to that taken from the tail is due to the longer time over which the anesthesia was allowed to act. This is supported by the fact that in a separate study red cell counts made on blood samples taken simultaneously from the tail and heart of a group of ten anesthetized rats were nearly identical, the means being 7,651,000 and 7,513,000 respectively. Similar counts on a group of ten unanesthetized rats also showed the blood from these two sources to be nearly identical, the numbers being 8,967,000 and 8,790,000.

TABLE I.
Effect of Anesthesia by Nembutal and by Ether on the Erythrocyte Number of the Blood of the Albino Rat. 20 Rats for Each Determination.

Treatment and source of blood	Red blood cells: Mean* × 1000	Red blood cells: Range × 1000
No anesthesia; tail	8,530 ± 130	(7,220-9,780)
Nembutal; tail	7,802 ± 109	(6,430-8,650)
" heart	7,376 ± 136	(6,330-8,180)
No anesthesia; tail	8,368 ± 200	(7,440-9,384)
Ether; tail	7,665 ± 210	(7,106-8,926)
" heart	6,965 ± 88	(6,240-7,910)

TABLE II.
Effect of Anesthesia by Nembutal and by Ether on the Specific Gravity of the Blood of the Albino Rat. 20 Rats for Each Determination.

Treatment and source of blood	Specific gravity: Mean*	Specific gravity: Range
No anesthesia; tail	1.0603 ± .0002	(1.059-1.062)
Nembutal; tail	1.0549 ± .0003	(1.053-1.058)
" heart	1.0529 ± .0003	(1.051-1.055)
No anesthesia; tail	1.0584 ± .0003	(1.058-1.061)
Ether; tail	1.0548 ± .0002	(1.053-1.057)
" heart	1.0521 ± .0001	(1.050-1.055)

* Including standard deviation of the mean.

[8] Gibson, J. G., 2nd, and Branch, C. D., *Surg., Gyn. and Obstet.*, 1937, **65**, 741.

[9] Ragan, C., Ferrebee, J. W., and Fish, C. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 712.

[10] Conley, C. L., *Am. J. Physiol.*, 1941, **132**, 796.

[11] Barbour, H. G., *Anesthesiology*, 1940, **1**, 121.

[12] Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Jr., Hamilton, P. B., and Archibold, R. M., *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma*, Josiah Macy, Jr., Foundation, New York, Feb., 1945.

Summary. Anesthesia in the rat by means of nembutal or ether produces a hemodilution, as shown by both a decrease in the red cell numbers and in the specific gravity of the blood.

16350

Possible Eczematous Cross-Hypersentitivity Between Paraphenylenediamine and Azo-Dyes Certified for Use in Foods, Drugs and Cosmetics.*

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In recent reports Dobkevitch and Baer[1,2] have analyzed in a number of patients the cross-sensitization between paraphenylenediamine and certain azo-dyes used in the manufacture of nylon stockings. It was found that this cross-sensitization represented a new example of the complex of hypersensitivity to compounds of quinone structure, as described by Mayer.[3,4] The present study was undertaken to ascertain, in subjects with allergic eczematous contact-type sensitization to paraphenylenediamine, whether this cross-sensitization extends not only to those azo-dyes used in the dyeing of various goods or as therapeutics for dermatological purposes, but also to those azo-dyes which are commonly used in the United States as dyes, for foods, drugs and cosmetics.

The use of synthetic dyes in foods, drugs and cosmetics is regulated under authority of the Federal Food, Drug and Cosmetic Act of 1938 and no coal tar colors other than those listed and described in these regulations may be used in foods, drugs and cosmetics. Among the 116 dyes listed, 18 are accepted for use in foods, 10 of which are azo-dyes. These 10 dyes are "straight" colors, free from all impurities other than a specified maximal allowance (5%) for chlorides and sulfates of sodium; for certain aromatic amines, as o-toluidine, 0.05%, permitted in FD&C Orange No. 1; or xylidine, 0.1%, in FD&C Red No. 32; for certain aromatic hydrocarbons, as betanaphthol, 0.05% in the above-mentioned dyes; and permitting only traces of metals such as arsenic (0.00014%) and lead (0.001%).

All colors used in foods have been examined by the Food and Drug Administration and found to be harmless in the toxicologic sense. The present regulations[5] do not set any standards for ascertaining the eczematogenic potential of dyes to be used in foods, drugs and cosmetics, although all of the certified colors have been tested for sensitizing capacity on the skin of groups of guinea pigs (Calvery[6]).

Experimental. Twenty-five subjects with known allergic eczematous contact-type hypersensitivity to paraphenylenediamine and 21 control subjects with no hypersensitivity to this compound but most of them with hypersensitivity to other non-related substances were given patch tests with the following substances:

* With the technical assistance of Mrs. Dorothy Furman.

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1 Dobkevitch, S., and Baer, R. L., *J. Invest. Dermat.*, 1947, **8**, 419.

2 Dobkevitch, S., and Baer, R. L., *J. Invest. Dermat.*, 1947, **9**, 203.

3 Mayer, R. L., *Arch. f. Dermat. u. Syph.*, 1928, **156**, 312.

4 Mayer, R. L., *J. Invest. Dermat.*, 1948, **10**, 389.

5 Federal Security Agency, Food and Drug Administration: Coal-Tar Color Regulations, September, 1940.

6 Calvery, H. O., *Am. J. Pharm.*, 1942, **114**, No. 9.