

Dehydration must be rapid so that cyton areas do not become completely destained. After washing in 80 per cent. alcohol the sections will become reddish in color with blue cyton areas. If the dehydration is properly carried out, the following structures will be selectively and permanently stained:

Cytons and Nissl granules deep blue; nuclei of blood vessel structures and neuroglia light blue; elastic fibers of blood vessels deep blue; erythrocytes pink; and neuroglia fibers light red.

The intensity of the cyton stain may be increased or decreased by varying the proportion of the Giemsa solution in the mixture.

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A RAPID METHOD FOR REMOVING COVER GLASSES OF MICROSCOPE SLIDES

It is often necessary in cytological work to remove the cover glass of a slide, in order to replace a broken

cover glass or restain the sections underneath. For this purpose most workers use xylene. The writer, however, has found that a mixture of 90 parts of xylene and 10 parts of n-butyl alcohol acts much more rapidly. The hard and brittle balsam or damar of old slides, which would require an immersion of several days in pure xylene, is usually dissolved by this mixture in a few hours. This time difference is probably due to the presence—especially in old slides—of a small amount of moisture in the mounting medium around the margin of the cover glass. Such moisture would offer a barrier to the penetration of pure xylene, but not to xylene containing n-butyl alcohol; for the latter is miscible with small amounts of water, as well as with xylene, balsam and damar. It should be remembered that butyl alcohol is a solvent of the aniline dyes, and so material stained with these substances will be destained in this xylene-butyl alcohol mixture.

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SPECIAL ARTICLES

X-RAY DIFFRACTIONS FROM HEMOGLOBIN AND OTHER CRYSTALLINE PROTEINS

SEVERAL attempts have been made during the past years to get x-ray diffraction photographs of the crystalline proteins. Most of these¹ have not been successful, but in a few instances very simple patterns have been observed.² These patterns, which always consisted of two rather broad and diffuse rings, have been found from proteins as different as edestin, excelsin, egg albumin and hemoglobin.

The diffuseness of the rings, combined with their simplicity irrespective of diffracting substance, suggests the pattern of a glass or other amorphous material rather than of a crystal. The probability that they are such amorphous patterns is strengthened by the recent statement³ that a typical sharp line pattern can be prepared from a single crystal of pepsin left in its mother liquor.

We have been seeking to obtain truly crystalline powder patterns from edestin, excelsin and hemoglobin. Photographs prepared in the usual way from (1) commercial edestin, (2) well-crystallized edestin and excelsin freshly made from hemp seeds and Brazil nuts and (3) crystalline (white rat) oxy- and carbon

monoxy-hemoglobin gave the familiar "amorphous" bands. When these preparations were examined microscopically they proved to be more or less completely altered after photography. Further microscopic study demonstrated that the protein crystals always decomposed rapidly on exposure to air. From the way this disintegration took place it was clear that they all contained water of crystallization which was very readily lost.

Photographs with copper K radiation were accordingly made of the wet crystals sealed into thin containers having windows of 0.01 mm glass. Under such conditions the protein crystals remain unchanged and typically crystalline patterns, consisting of fine, though very faint, lines, are produced. With this experimental arrangement reflections corresponding to large spacings lie too close to the central image for accurate measurement. Additional, and far more instructive, photographs have consequently been made with the longer chromium K radiation by keeping samples in moist chambers without protective windows. Some spacings thus measured on typical pictures of rat oxyhemoglobin are listed in Table I. There is no reason to believe that we have yet established the largest spacings that exist for this hemoglobin or for the other protein crystals. Our techniques, however, are being improved and it is expected that these maximum spacings will eventually be determined.

¹ For example, R. O. Herzog and W. Janeke, *Naturwiss.*, 9: 320, 1921; W. H. George, *Proc. Leeds Phil. Soc.*, 1: 412, 1929.

² See J. R. Katz, "Die Röntgenbeugungsphotographie als Untersuchungsmethode" (Berlin, 1931), p. 188.

³ J. D. Bernal and D. Crowfoot, *Nature*, 133: 794, 1934.

TABLE I
SPACINGS OF SOME POWDER LINES OF OXYHEMOGLOBIN

Spacing	Estimated intensity	Spacing	Estimated intensity
45.7 Å	s (broad)	10.4 Å	m
27.5	m	9.4	f
21.7	m -	8.4	f
18.0	ff	5.93	ff
15.4	f	4.90	ff
13.0	m	4.62	f
11.6	ff	3.47	ff

In this table s, m, f, ff represent strong, medium, faint and very faint. It is possible that one or two of the fainter lines are beta reflections.

In the meantime it may be concluded that:

(1) The proteins edestin, excelsin and hemoglobin crystallize with water of crystallization, which is very readily lost. The band pattern previously described as common to these and other proteins is produced by apparently amorphous materials resulting from the efflorescence of the crystalline compounds.

(2) If care is taken to prevent the decomposition of their crystals, these proteins give typical powder patterns rich in sharp lines. Some of the observed spacings are much longer than those found from insoluble protein structures like silk, hair and tendon.

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EFFECTS OF THEELIN ON THE MALE GENITAL TRACT

THE principal known action of theelin (ketohydroxyoestrin) is to induce tissue proliferation in the accessory genital organs of the female. This has been demonstrated in the usual laboratory animals; the monkey and man. Since an oestrus-inducing agent has been extracted from the urine of normal human males¹ it seemed desirable to study the action of theelin on the male genital tract. Some changes have been described in the mouse² and rat³; and in the male monkey it is known that this oestrogenic extract is responsible for a sexual skin reaction.

The effects of interest here have been brought about by the subcutaneous injection of 60 cc of theelin⁴ over

¹ E. Laqueur, E. Dingemans, P. C. Hart and S. E. de Jongh, VI Mitteilg *Klin. Wchnschr.*, 6: 1859-1868, 1927.

² Harold Burrows and N. M. Kennaway, *Am. Jour. Cancer*, 20: 48-57, 1934.

³ John Freud, *Biochem. Jour.*, 27: 1438-1450, 1933.

⁴ Through the courtesy of Dr. Oliver Kamm, of Parke, Davis and Company, we have received theelin for this study.

a period of thirty-four days into an immature male monkey weighing 2,450 gms at autopsy. A cage mate of similar age, weighing 3,000 gms at autopsy, was used as a control. The most striking change in growth was found in the seminal vesicles, those of the injected animal weighing 5½ times as much as those of the control. Histological study showed the increase entirely due to muscular hypertrophy of the walls of the vesicles. There had been no stimulation of the secretory epithelium, and the lumen of the individual tubule had fewer outpocketings than the control. It appears that the activity within the wall had restricted concurrent increase in lining epithelium. The ejaculatory ducts were enlarged. Within the prostate there was also a relative increase in fibromuscular stroma at the expense of the epithelial glands. As in the prostate of the adult castrate monkey, where the epithelium is markedly degenerated, the prostate takes a more posterior position in relation to the urethra. Another striking tissue change was found in the prostatic utricle where extensive cornification of the epithelium had taken place increasing the thickness of the epithelium as much as twenty-five times.

The prostatic utricle is a remnant of the Müllerian ducts and as such the change here is analogous to the well-known effect in the vaginal mucosa. In view of this the report of a similar effect² in the posterior prostatic lobes of the mouse may indicate that these lobes are not true prostate but have an origin in common with the utricle. In the monkey this cornification extended along the posterior wall of the urethra into the membranous portion and the columnar epithelium of the pars cavernosa had become cornified.

Notwithstanding the great development of the scrotal sac the right and left testes (which had not increased in size) lay in the groin 3.5 cm and 2.5 cm, respectively, from the external inguinal rings. There was incontinence of urine and the usual swelling of superficial tissues about the distal portions of the genital tract.

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BOOKS RECEIVED

- DALAKER, HANS D. and HENRY E. HARTIG. *The Calculus*. Third edition. Pp. viii + 276. 107 figures. McGraw-Hill. \$2.25.
- HALL, SIR DANIEL and others. *The Frustration of Science*. Pp. 144. Norton. \$2.00.
- HERTY, C. H., JR. and others. *The Physical Chemistry of Steel Making*. Illustrated. Mining and Metallurgical Advisory Boards to the Carnegie Institute of Technology. \$3.00.
- KNOWLTON, A. A. *Physics for College Students*. Second edition. Pp. xxi + 623. Illustrated. McGraw-Hill. \$3.75.
- MCCOMBS, LOIS F. and MORRIS SCHRERO. *Bibliography of Non-Metallic Inclusions in Iron and Steel*. Pp. xii + 308. Mining and Metallurgical Advisory Boards to the Carnegie Institute of Technology. \$4.00.