STANDARDIZED NOMENCLATURE OF BIOLOGICAL STAINS

AT a meeting of manufacturers and dealers in biological stains in September, 1922, the committee in charge of the investigation of stains was asked to draw up a standardized list of the most commonly used stains indicating in each case both the preferred designation and the most commonly used synonyms. Such a list has now been prepared with the cooperation of the manufacturers and others interested in these stains. This list is hereby published as a step toward stabilizing the nomenclature of these products.

The list as given here is divided into two groups: first the stains which might be called staples and which are necessary in practically all biological laboratories; and secondly the less commonly used stains which are required only for special purposes. Manufacturers and dealers assure us that the bulk of the business is done with the stains on the first of these two lists.

The accompanying list of stains is not claimed to be anywhere near complete. There are many dyes sometimes used as stains for special purposes that are not included because

LIST OF ORDINARY STAINS

I. STAPLES

(a) *Synthetic dyes*

Schultz No.	Preferred Designation	Synonyms; and slightly varying shades
38	Orange G	Patent orange. Crystal orange. Wool orange. (Slightly differing grade: Orange GG, GMP)
124	Janus green B	Diazin green.
223	Sudan III	Scarlet G or B. Fettponceau G. Oil red. Cerasin red.
307	Congo red	Congo. Cotton red. Direct red. Cosmos red.
511-3*	Fuchsin, basic	Fuchsin. Diamond fuchsin. Magenta. Rubin. Rosanilin. Rosanilin hydrochloride. (Different grades sold under the name of fuchsin followed by various shade designations).
515	Methyl violet 2B	Methyl violet. Dahlia B. Paris violet. Gentian violet. Pyoktanin blue. (Slightly differing grades: Methyl violet B, BBN, BO and V3).
516	Crystal violet	Violet C, G or 7B. Gentian violet. Hexamethyl violet.
.....	Gentian violet†	
519	Methyl green	Double green. Light green.
524	Fuchsin, acid	Fuchsin S, SN, SS, ST or S III. Acid magenta. Acid rubin.
539	Anilin blue, water soluble	China blue. Soluble blue. Marine blue. Blackley blue. Cotton blue. Water blue. Berlin blue.
568	Pyronin	
587	Eosin, yellowish	Eosin. Water soluble eosin. Eosin Y, W or WS. (Various grades denoted as Eosin G, Y extra, S extra, J extra, B extra, GGF, 3J, 4J, KS, DH and JJF.
659	Methylene blue, Med., U.S.P.	Swiss blue. (Slightly different grades: Methylen blue BX, B, BG and BB).
670	Neutral red	Toluylene red.
679	Safranin O.	Safranin AG, T, MP, Y and G.

(b) *Natural dyes*

932	Cochineal	(The aqueous extract of the cochineal insect).
	Carmine	(The lake prepared by adding alum to cochineal).
	Carminic acid	(The active dye purified from cochineal).
938	Hæmatoxylin	(The ether extract of logwood).
	Hæmatein	(The dye formed on oxidation of hæmatoxylin).

* Apparently three different dyes, corresponding to Schultz numbers 511, 512 and 513, are sold as basic fuchsin. It is apparently the case that a different product is required as an indicator in the Endo medium from that required for staining; but conflicting statements have been received, and the matter needs investigation.

† Nearly synonymous with methyl violet; various mixtures of methyl and crystal violets sold under this name, generally with a high percentage of dextrin.

the call for them is small, although in some cases new developments in technic may cause a growing demand for them in the future. For this reason manufacturers and dealers in stains are not asked to list no other stains in their catalogs; but it is hoped that so far as their catalogs include the stains given in the accompanying list they will use the nomenclature here recommended. It is suggested that they use the two lists given here, with the Schultz number and as many synonyms as they care to employ and then as a supplementary list those stains which they have in stock but which are not included in this list.

In explanation of this list it must be remarked that wherever possible only one shade of any particular stain has been listed and the closely related shades are mentioned in the column of the table indicated for that purpose. This is done because in most cases it proves that only one or two of the various shades are of any special use as biological stains. It should further be explained that the Schultz number given in the first column refers to the fifth edition of Schultz's *Farbstofftabellen* published in 1920, except in the case of the last few stains in the table, which were listed in earlier editions of this publication but not in

II. LESS COMMONLY USED STAINS

Schultz No.	Preferred Designation	Synonyms; and slightly varying shades
4	Naphthol green	
6	Martius yellow	Naphthol yellow. Naphthalene yellow. Manchester yellow.
33	Chrysoidin Y	Chrysoidin. Brown salt R. Dark brown salt R. (Slightly different shades: Chrysoidin A, G, 2G, R, J, RE, JEE).
34	Chrysoidin R	Chrysoidin. Cotton orange. Cerotin orange. (Slightly different shades: Chrysoidin REE, RG).
112	Bordeaux red	True red B, P. Cerasin. Archelline 2B. Azo-bordeaux. (Various grades denoted as: Bordeaux B, BL, G, R extra).
138	Methyl orange	Orange III. Helianthin. Gold orange.
139	Orange IV	Orange N. Acid gold D. Tropæolin OO. Citronin V.
144	Orange I	Naphthol orange. Orange S. Tropæolin G, OOO No. 1.
145	Orange II	Gold orange. Orange A, P, G, R. Acid orange. Orange extra. Mandarin G. Tropæolin OOO No. 2.
168	Amaranth	Naphthol red. True red. Bordeaux. Bordeaux SF. Victoria rubin. Azo rubin. Wool red.
232	Sudan IV	Scarlet red. Fettponceau. Ponceau 3B.
247	Biebrich scarlet, water soluble	Ponceau B. Double scarlet. Scarlet EC.
283	Bismarck brown Y	Bismarck brown. Vesuvin. Phenylene brown. Manchester brown. Excelsior brown. Leather brown. (Slightly different shade: Bismarck brown G).
359	Trypan red	
363	Benzopurpin 4B	Cotton red. Dianil red. Diamin red. Sultan 4B. Direct red 4B.
391	Trypan blue	Diamin blue. Benzo blue. Dianil blue. Congo blue. Naphthamine blue. Benzamin blue. Azidin blue. Niagara blue.
493	Auramin	Pyoktaninum aureum. Pyoktanin yellow.
495	Malachite green	Emerald green. New Victoria green. Diamond green. Solid green. True green. New green. Light green N.
499	Brilliant green	Ethyl green. Solid green. Benzaldehyde green. Malachite green G.
505	Light green SF, yellowish	Light green 2G, 3G, 4G, 2GN. Acid green D, G, 5G, H, F, GB, GG, G extra, GW, OOO, O yellowish.
514	Hoffman violet	Dahlia. Iodine violet. Primula R. Red violet. Violet, R, RR, 4RN.
521	Spirit blue	Anilin blue, alcohol soluble. Gentian blue. Night blue. Lyons blue. Paris blue.
536	Alkali blue	
538	Methyl blue	Cotton blue. Wool blue. Helvetia blue.
555	Rosolic acid	Aurin.
555	Corallin, yellow	Sodium salt of rosolic acid.

the fifth edition. In such cases as this both the edition and the list number in that edition are given.

The list as it now stands is to be regarded as a preliminary one, subject to modification. In spite of all the advice received in preparing it, there are probably points in it still that are not correct. It is also obvious that the choice of preferred designation and the placing of any stain in either the first or the second list was more or less arbitrary and had to be a compromise between the various opinions re-

Schultz No.	Preferred Designation	Synonyms; and slightly varying shades
573	Rhodamin B	Rhodamin O. Brilliant rose.
585	Fluorescein, U. S. P.	Uranin.
588	Eosin, alc. sol.	
590	Eosin, bluish	Eosin BN, B, BW, DHV. Methyl eosin. Safrosin. Eosin scarlet B, BB. Scarlet J, JJ, V. Nopalin G. Cæsar red.
592	Erythrosin, bluish	Erythrosin B. Pyrosin B. Eosin J. Iodo-eosin. Dianthine B. (Slightly different shades: Erythrosin D, J, JNV, W).
597	Rose bengal	(Various grades denoted as: Rose bengal B, 2B, 3B).
621	Brilliant cresyl blue	Cresyl blue. Cresyl blue 2RN or 2BS.
653	Nile blue sulfate	Nile blue A.
694	Magdala red	Naphthalene red. Naphthylamine red.
697	Indulin, alc. sol.	Acetin blue. Azin blue. Solid blue.
698	Nigrosin, alc. sol.	Nigrosin B, BB.
699	Indulin, water sol.	(Various grades denoted: Indulin NN, N, N6, B, L, etc.).
700	Nigrosin, water sol.	Anilin gray. Steel gray. Indulin black. Gray R, B, BB. (Various grades denoted: Nigrosin DW, R, G, etc.).
777	Chromogen I	
788	Alizarin	(Various grades denoted as: Alizarin P, VI, Ie).
780	Alizarin red S.	Alizarin monosulphonate of sodium. Alizarin red WS. Alizarin carmine.
877	Indigo carmine	Indigotine.
934	Orcein	
I 276*	Lacmoid	Resorcin blue.
II 348	Thionin	Lauth's violet. (*Not* thionin blue, which is Schultz No. 661).
II 284	Iodine green	
IV 592	Toluidine blue O	
.....	Azur I†	Methylene azur.
.....	Azur II†	
.....	Methylen violet, Bernthesen	(*Not* methylen violet RRA or 3RA, Schultz No. 680).
.....	Cresyl violet	Cresyl echt violet.

* The Schultz number refers ordinarily to the 5th edition of Schultz' Farbstofftabellen. When the number is preceded by a Roman numeral, the dye in question is not listed in the 5th edition and the Roman numeral indicates the edition in which it is to be found.

† See U. S. Pharmacopœia, 9th Edition, p. 525.

III. COMPOUND STAINS*

Name	Reference
Azur-II-eosin	U. S. Pharmacopœia, 9th Edition, p. 525.
Balch's blood stain	Stitt, Practical Bacteriology, 6th Edition, p. 261.
Biondi-Ehrlich-Heidenhain triple mixture	Lee, Microstomists Vade-Mecum, 8th Edition, p. 173.
Ehrlich's triacid or triple mixture	Stitt, 6th Edition, p. 260.
Jenner's stain	Lancet, 1899, p. 370.
Leishman's blood stain	Stitt, 6th Edition, p. 261.
Tetrachrome blood stain (MacNeal)	Zinnser, Bacteriology, 4th Edition, p. 797.
Pappenheim's stain	Stitt, 6th Edition, p. 54.
Pappenheim's panoptic triacid stain	Lee, 8th Edition, p. 383.
Wright's blood stain	Mallory and Wright, Pathological Technic, 5th Edition, p. 364.

* The compound stains listed here are those which are commonly sold already compounded by manufacturers and dealers in stains, either in dry or in liquid form.

ceived. The future may show the desirability of changing some of these matters. Any suggestions along this line by readers of this article will be gratefully received.

It is hoped that both those who use and those who manufacture or handle biological stains will cooperate in this standardization, the latter by using the nomenclature in their catalogs and price-lists and the former by ordering stains by the names given here, specifying the Schultz number whenever one is listed.

COMMISSION ON STANDARDIZATION OF BIOLOGICAL STAINS.
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SPECIAL ARTICLES

REVERSE MUTATION OF THE BAR GENE CORRELATED WITH CROSSING OVER

THE bar gene of Drosophila melanogaster reverts to its normal or wild-type allelomorph in from thirty to sixty individuals per hundred thousand (May 1917, Zeleny 1918, 1921, 1923). This is a far higher frequency of mutation than is shown by any other locus in Drosophila. We have now found that bar reversion shows a relation to crossing over in the bar region.

Zeleny (1921) found that bar reversions usually occur singly, so that it is not probable that the mutation occurs far back in the history of the germ-cells. It has been the experience of all who have studied bar stocks that in any given specimen the gonads and the eyes are of the same constitution—that is, that a bar-eyed fly breeds as a bar, and a round-eyed fly breeds as a not-bar. These facts further indicate that the bar reversion occurs either late in the history of the germ-cells, or else before the first cleavage division.

If reversion occurs in the germ-cells of both sexes with equal frequency homozygous stocks should produce twice as many heterozygous bar females as wild-type males; if it occurs only in females the number of heterozygous females produced should be equal to the number of wild-type males; if it occurs only in males the reverted offspring should all be heterozygous females. Zeleny (1923) has obtained from homozygous bar stocks a total of fifty-two heterozygous bar females and fifty wild-type males, thus indicating that reversion occurs only (or chiefly) in the germ-cells of females. We can confirm the absence of reversion in males, from extensive experiments in which bar males were mated to not-bar females, without the production of a single reversion.

In an attempt to determine whether the reversion of bar affects one or both chromosomes of a homozygous female, the following experiment was carried out. Females were made up that were homozygous for bar and for the sex-linked recessive eye-color eosin, and that carried a Y-chromosome. These females were also made up in such a way as to give "high" non-disjunction. Such females were mated to vermilion (not-bar) males. Disregarding gynandromorphs, mutations other than bar reversion and a few anomalous results probably due to non-virginity or to mutation, the following counts were obtained by Miss E. M. Wallace:

REGULAR OFFSPRING

red heterozygous bar ♀	eosin bar ♂	red not-bar ♀	eosin not-bar ♂
46518	40303	19	8

Percentage of reversions, .03

EXCEPTIONAL OFFSPRING

eosin homozygous bar ♀	vermilion not-bar ♂	eosin heterozygous bar ♀
12223	11440	1

Percentage of reversions, .008

These results, taken together with the facts previously outlined, at once suggested that bar reversion has something to do with crossing over, since the most striking difference between the regular and exceptional offspring of an XXY female is that the former include almost or quite the normal proportion of crossovers, while the latter are only very rarely crossovers (Bridges 1916). On this view the one eosin heterozygous bar female produced might be interpreted as an "equational" exception (in which case the experiment was inconclusive for the purpose for which it was intended).

The crossover suggestion has now been verified directly in two separate experiments. The two sex-linked recessives forked (f, locus 56.5) and fused (fu, locus 59.5) lie on opposite sides of the bar locus, but are only about three units