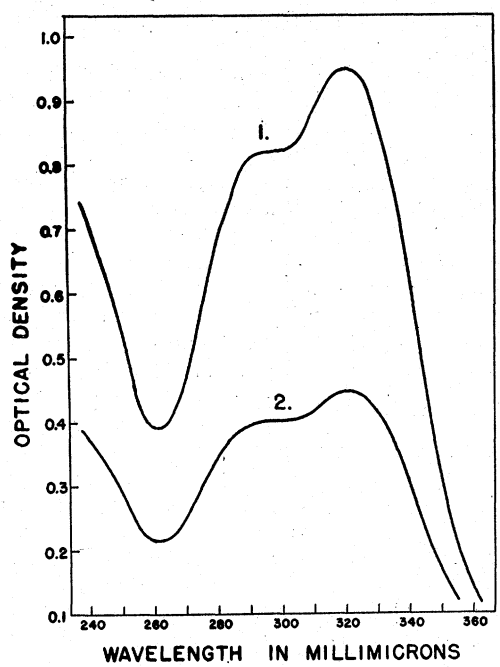

FIG. 4. UV absorption spectra of the peeling extract from a scab-resistant seedling (1) and from a scab-susceptible seedling (2).

curves likewise show much higher concentration of chlorogenic acid in the scab-resistant seedlings as compared to a scab-susceptible seedling.

The total amount of chlorogenic acid is not as important as the concentration in local areas. For example, in some scab-resistant potato varieties, the chlorogenic acid appears to be more heavily concentrated in or near the lenticels, which serve as the natural avenue of entrance for the scab organism. The $FeCl_3$ test also indicates that chlorogenic acid accumulates around a tissue injury, either mechanical or parasitic in origin. This observation, together with the fact that the concentration of the acid in the periderm varies with the degree of scab resistance, suggests that chlorogenic acid is involved in a protective mechanism. The exact nature of this protection has not been determined, but indications are that one or more mechanisms are involved.

It is possible that chlorogenic acid lowers pH of the cells, thereby creating an unfavorable medium for growth of the scab organism. Experiments indicate that when chlorogenic acid was added to unbuffered potato dextrose agar medium, the lowered pH was sufficient to retard growth of *S. scabies*. On buffered agar (pH 6.2) 400 mg chlorogenic acid/100 ml agar failed to retard growth of several physiologic races of *S. scabies*.

Since tyrosinase is also concentrated in the same area as chlorogenic acid, it may be that both are involved in a general protective mechanism. Chlorogenic acid is a good substrate for the polyphenolase fraction of tyrosinase. Upon tissue injury tyrosinase immediately oxidizes chlorogenic acid to the quinone which may be toxic to pathogenic organisms, and this would be in accord with views expressed by Szent-Györgyi and Vietorsz (*9*) as to a function of tyrosinase. The rate of quinone formation would vary with the concentrations of chlorogenic acid and tyrosinase. Quinone formation in the area where chlorogenic acid was concentrated was also found to be greater in the potato varieties resistant to scab. An acidified KI-starch solution was used as a test for quinone formation after macerating the tissue with a sharp knife.

Chlorogenic acid itself or its quinone may be directly or indirectly involved in the formation of cork cambium in a manner similar to action of the wound *hormone* traumatin as described by Bonner and English (*10*). This is believed true because chlorogenic acid added to a freshly cut surface of a tuber speeds up the production of suberized tissue.

References

1. LUTMAN, B. F., and CUNNINGHAM, G. C. *Agr. Expt. Sta. Bull. Vermont,* **184,** (1916).
2. LONGREE, K. *Arb. biol. Reichsanstalt Land-u. Forstw. Berlin-Dahlem,* **19,** 285 (1931).
3. KIESSLING, L. E. *Zentr. Bakt. Parasitenk.,* Abt. II, **89,** 177 (1933).
4. WINGERBERG, F. *Kühn-Arch.,* **33,** 259 (1932).
5. MÜLLER, K. O., and BEHR, L. *Nature,* **163,** 498 (1949).
6. WALKER, J., *et al. Proc. Natl. Acad. Sci. U. S.,* **15,** 845 (1929).
7. JOHNSON, G., *et al. Food Research,* **16,** (3), 169 (1951).
8. MOORES, R. G., *et al. Anal. Chemi.,* **20,** 620 (1948).
9. SZENT-GYÖRGYI, A., and VIETORSZ, K. *Biochem. Z.,* **233,** 236 (1931).
10. BONNER, J., and ENGLISH, J. *Plant Physiol.,* **13,** 331 (1938).

Manuscript received January 3, 1952.

A Modification of the Sudan Black B Technique for the Possible Cytochemical Demonstration of Masked Lipids[1, 2]

G. Adolph Ackerman

Departments of Anatomy and Medicine, The Ohio State University, Columbus

The technique used in the study described here is an adaptation of a routine cytochemical method employed on blood films to demonstrate lipids. This modification is designed to reveal what are commonly called "masked" lipids not demonstrable by routine procedures. It involves the common use of Sudan black B, but on films previously treated with various organic acids.

The routine procedure for the demonstration of masked lipid in blood films is as follows:

1. Fix smears in formol vapor for 2–5 min.
2. Immerse fixed films in a 25% aqueous solution of acetic acid for 2 min. Citric acid (5%), oxalic acid (10%), or formic acid (10–25%) may be used in place of acetic acid.

[1] This investigation was supported by research grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and the Ohio Department of Health.
[2] Presented in part before The Ohio Academy of Science, April 28, 1950.

3. Wash thoroughly in tap water; wash in distilled water and allow films to dry.

4. Place dried films in a saturated solution of Sudan black B in 70% ethyl alcohol for 30 min. The Sudan black solution must be prepared at least 1 week prior to use.

5. Wash stained films with either 50% or 70% ethyl alcohol until excess Sudan black is removed. Wash immediately with water.

6. Blot films dry and mount with permount, glycerine, or glycerogel. Parallel control preparations are processed in the same manner except that the treatment with acid is omitted.

Fixatives successfully adapted for brief fixation of blood films and the demonstration of masked lipids are heat, formol vapor, osmic acid vapor, 10% formalin, formol-Zenker, mercuric chloride (1%), copper sulfate (0.5%), 1% formalin in 95% ethyl alcohol, methyl alcohol, and acetone. Bouin's solution, trichloracetic acid (20%), cobaltous nitrate (1%), and uranium nitrate (1%) should not be used in this procedure. Formol vapor is considered the fixative of choice because of the ease of fixation and also because it may be used as the fixative for a wide variety of cytochemical procedures performed on blood films.

Lipids have been unmasked by four carboxylic acids (formic, acetic, citric, and oxalic acids), but cannot be unmasked by mineral acids (1 *N* hydrochloric, 1 *N* sulfuric, 1 *N* nitric, 0.5% periodic, and carbonic acids). Hydrochloric acid prepared at pH 1, 2, 3, 4, 5, and 6 failed to unmask any sudanophilic material in blood cells, and the more acidic solutions, pH 1 and 2, prevented the lipid normally demonstrated in control preparations from staining. Weak bases (saturated solutions of calcium hydroxide and lithium carbonate and 10% ammonium hydroxide) and strong base (1 *N* sodium hydroxide) failed to unmask cellular lipid.

Sudan black B prepared in 70% ethyl alcohol, 40% ethyl alcohol, ethylene glycol, and 50% acetone may be used in the procedure described, although it must be noted that the staining reaction and color of control preparations is different, depending upon the solvent used.

TABLE 1

EFFECT OF VARIOUS LIPID SOLVENTS AND PROTEOLYTIC ENZYMES ON THE NUCLEAL AND MODIFIED SUDAN BLACK REACTIONS

Solvent and enzyme	Nucleal reaction	Acetic acid–Sudan black reaction
Control	++++	++++
Alcohol-ether (2:1)	++++	–
Acetone	++++	+
Desoxyribonuclease (0.1 mg %)	–	–
Trypsin (1%)	+	+
Pepsin (1% in 0.1 *N* HCl)	±	+
Sodium chloride (1 *M*)	++	++
Distilled water	+++	+++

+ Indicates the relative intensity of the reaction following digestion for 24 hr in the above substances as compared with undigested control (++++) preparations.
– Indicates no reaction.

A comparison of blood films stained with Sudan black B in 70% ethyl alcohol (control) and films treated with acetic acid prior to staining with Sudan black reveals marked differences. The most striking differences are: (1) in acid-treated preparations the nuclei of the leucocytes and nucleated erythrocytes are sudanophilic, staining brown to yellow-brown, in contrast to their sudanophobic nature in control Sudan preparations; (2) platelets only slightly sudanophilic in control preparations are moderately sudanophilic (brown with blue-black granules) in acid-treated films; (3) black sudanophilic granules of neutrophils and monocytes in control preparations are not evident in acid-treated films, although many small sudanophilic granules are unmasked in lymphocytes and monocytes; (4) blood plasma appears slightly sudanophilic in acid-treated films, but is not evident in control preparations; (5) erythrocytes are rendered sudanophobic following acid treatment.

Cohen (*1*) found that cell nuclei could be stained with Sudan black to which organic acids were added; he believes that the organic acid dye solutions are simply nuclear stains. Leach (*2*) considers the brown dye formed by the addition of diacetin to Sudan black ("mucisudan") to be a stain for mucin.

Table 1 illustrates the effect of various proteolytic enzymes and lipid solvents on the sudanophilic and desoxyribonucleoprotein components of the cell nucleus as demonstrated by the acetic acid–Sudan black technique and the nucleal reaction, respectively.

The lipid nature of the sudanophilic material is suggested by the solubility of this component in alcohol-ether, hot pyridine, and partial solubility in acetone, as well as the insolubility of the desoxyribonucleoprotein with these solvents. The possibility of the lipid existing as a lipoprotein complex, perhaps a liponucleoprotein complex, is indicated by the solubility of the sudanophilic component by various proteolytic enzymes, including desoxyribonuclease. It was impossible to unmask the sudanophilic material of the cell nuclei with the substances indicated in Table 1.

Further evidence supporting the view that lipids are unmasked and demonstrated by the technique described include the following observations: (1) Biochemical studies (*3*) on nuclei reveal that they contain considerable quantities of lipid, principally phospholipids (sphingomyelins or saturated lecithins or cephalins) and cholesterol. The phospholipid content of the nuclei is suggested by this Sudan technique and the extraction experiments. (2) Platelets contain a considerable amount of phospholipid (15% dry weight), principally cephalin. Phospholipid and plasmalogen may be demonstrated cytochemically in blood platelets as determined by Baker's acid haematin test (*4*) and the plasmal reaction (*5*), respectively. However, platelets, sudanophobic after staining with alcoholic Sudan black, may be readily demonstrated in organic acid–treated films. (3) The phospholipid content of lymphocyte mitochondria may be demonstrated

cytochemically (*5*); however, although lymphocyte mitochondria stain only very slightly with alcoholic Sudan black, following acid treatment their staining reaction is markedly enhanced. (4) Plasma lipids are unmasked by acid treatment, although a longer fixation time (i.e., 5 min) is necessary.

It is generally considered that lipids stain blue or black with a 70% alcoholic solution of Sudan black. Therefore, this technique may be criticized because a brown color is obtained in certain cellular components (nuclei and platelets) after acid treatment. This objection is minimized by the observations discussed above, as well as by several additional facts: (1) Sudan black prepared in 40% ethyl alcohol yields a brown solution; in 70% alcohol the solution is blue-black. Although the distribution and amount of sudanophilic material present in cells are identical after staining with these solutions, the color is different (brown with a 40% dye solution and black with a 70% dye solution). A similar color difference is obtained in the liposomes of the rat adrenal gland, as well as in adipose tissue (frozen sections). (2) The sudanophilic rim of the blood eosinophil granule is considered to be lipid (*4*), although it stains brown rather than black in control preparations stained with 70% alcoholic Sudan black.

The lipids of the mitochondria and blood platelets may be unmasked more readily using more dilute acid solutions than can the lipid of the nuclei. The differences in the color of the nuclei, platelets, and mitochondria following acid treatment and staining with Sudan black are suggestive of a difference in the type or form of phospholipid or lipoprotein complex. The mechanism by which lipid is unmasked by weakly ionizable acids is not known. However, the acids may act by dissociating or splitting the lipoprotein complexes and allowing the lipid to be accessible to the dye.

References

1. COHEN, I. *Stain Technol.*, **24**, 177 (1949).
2. LEACH, E. H. *J. Path. Bact.*, **47**, 637 (1938).
3. STONEBURG, C. A. *J. Biol. Chem.*, **129**, 189 (1939).
4. BLOOM, M. L., and WISLOCKI, G. B. *Blood*, **5**, 79 (1950).
5. ACKERMAN, G. A. Unpublished data.

Manuscript received December 31, 1951.

Comments and Communications

Common Names for Subspecies in Zoology

INEVITABLY, the science of one's own time seems somehow different in quality from the science of the past. No doubt Linnaeus's teacher, Olof Rudbeck the Younger, in the early 1700s, had the same feeling when he looked back on the science of Conrad Gesner; and Gesner himself must have felt the same way as he contemplated the works of Pliny. When we read the history of science with a discerning eye, we realize, perhaps with some surprise, that to students in the far future (if there are any) the apparently solid and sober structure of our contemporary science will be seen to be shot through with obvious errors and absurdities.

One of the latter—a minor one to be sure—will probably be the present fad of giving so-called common names (in reality, usually mere book names) to every subspecies of animal described by naturalists. The writer, be it understood, has no quarrel with standardized common names for easily recognizable and valid species. In a relatively few instances, such as that of the Common Canada Goose and the Cackling Goose, it would seem to be proper to assign common names even to subspecies. Neither does he question the necessity for giving *technical* names to valid subspecies. What he does object to as unnecessary and even ridiculous is the current fashion of publishing such names, to take a fanciful example, as Rufous-crowned Gray Dinglebat, Purple-sided Gray Dinglebat, Southern Plains Dinglebat, and Smith's Dinglebat for, let us say, four subspecies of critters which everyone has for generations called simply Gray Dinglebats, and which nobody but a specialist on dinglebats can tell apart anyway.

To take one real example, from the multitude available, in the serpent fauna of New Mexico *Pituophis catenifer* is known to all and sundry in my part of the country as the Bull Snake. In New Mexico there are three recognized subspecies of this snake—*P. sayi, P. affinis,* and *P. deserticola.* In the recently issued second edition of C. B. Perkins's *Key to the Snakes of the United States,* a standard reference work, I find these listed, respectively (p. 9), as Bull Snake, Sonoran Gopher Snake, and Great Basin Gopher Snake. Yet nobody but an ophiologist can tell them apart, and to the average English-speaking person in New Mexico they remain simply Bull Snakes. Biologists, likewise, almost always use the scientific names or just call the animals Bull Snakes. Possibly biologists farther west call them Gopher Snakes, but the principle is the same. Who, then, is supposed to use these complicated common names? And what about the individuals of *P. catenifer* in areas (extensive, be it noted) where *P. sayi* and *P. affinis* intergrade? If we accept the above-mentioned trinomial system of common names, these unlucky intergrading individuals are neither "bulls" nor "gophers" and presumably have no common name at all. Furthermore, turmoil is added to confusion when we note with dismay that Schmidt and Davis in their widely used *Field Book of Snakes of the United States and Canada* (p. 163) call Perkins' *P. c. affinis* the Arizona Bull Snake in-