PROBLEMS IN AERIAL APPLICATION:
HISTOCHEMISTRY OF WEIL STAIN ON LIVER

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I. Introduction.

The staining intensity of rat liver sections prepared by the Weil method for myelin sheaths is increased in endrin[1] and dieldrin[2] poisoning and decreased in cold adaptation[2]. Knowledge of the chemical nature of the compound or compounds involved might well be valuable in designing treatment for poisoning with these insecticides and possibly also in artificial cold adaptation. A step towards the characterization of these substances is available in the technics of histochemistry. In the spinal cord the Weil stain is apparently specific for some phospholipid containing a substituted amine[3]. Presumably the same should be true for the liver.

II. Methods.

The technics for acetylation, methylation, deamination and aldehyde blockage as given by Casselman[4] were rigorously followed. For trichloracetic acid extraction 0.3M acid at 90°C for 15 minutes was used and for amine blockage the ammonium salt of rhodanilic acid method of Berube, Powers and Clark[3]. In addition to the usual control an additional slide was placed in 0.5% paratoluene sulfonic acid (solvent used for the ammonium salt of rhodanilic acid) for the same length of time as in the blocking reaction. The variant of the Weil stain used was that developed by Berube, Powers and Clark[5]. Livers from rats perfused with acacia saline followed by acacia formalin fixation[6] were embedded in paraffin and sectioned at seven microns.

Also, small blocks of liver were rapidly excised from a rat killed by decapitation and placed in cold chloroform-methanol (2–1), pyridine, acetone, chloroform or methyl alcohol. These were kept at –20°C for one week and then the blocks were placed in 10% formalin for two days at room temperature. Control blocks were placed in 10% formalin for nine days at 3°C. These blocks were subsequently treated like unextracted blocks.

III. Results.

The results obtained are shown in the bar graph in Figure 1. In each case the corresponding results from similar treatment with spinal cord are given. These are taken from the report of Berube, Powers and Clark[3]. The results with spinal cord are shown as solid bars while those with liver are shown as striped bars. It is apparent that the intensity of the stain was increased with methylation, aldehyde blockage, acetylation, paratoluene sulfonic acid and trichloracetic acid extraction. Deamination for 30 minutes or 24 hours resulted in decreased staining intensity as did amine blockage with the ammonium salt of rhodanilic acid.

All the lipid solvents used markedly decreased the staining intensity of liver cytoplasm as shown

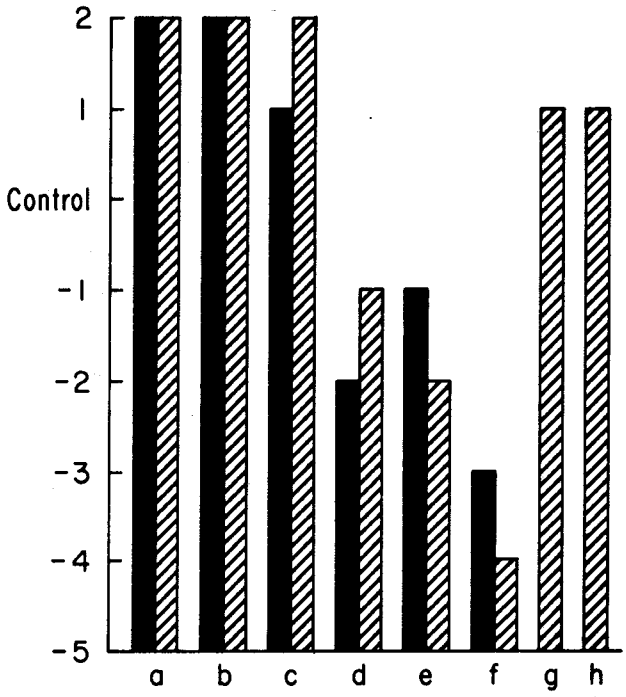

FIGURE 1. Bar graph showing staining intensity after various blocking procedures. Solid bars—spinal cord, striped bars—liver. a—methylation, b—aldehyde blockage, c—acetylation, d—amine blockage, e—deamination for 30 minutes, f—deamination for 24 hours, g—paratoluene sulfonic acid extraction and h—perchloric acid extraction.

in Table 1. Unexplicably with methyl alcohol, acetone and chloroform-methanol extraction, the cell membranes stained deeply and after acetone extraction red blood cells were intensely stained.

TABLE 1. Extraction at −20°C for one week then 2 days in 10% formalin. 10% formalin froze at −20°C so was kept at +3°C. 0, no stain, +, weak stain, ++, light stain, +++, normal stain, ++++, overstained.

	Cell Membranes	*Cytoplasm*	*RBC*
Chloroform-Methanol (2–1)	++++	+	lysed
Pyridine	0	0	lysed
Acetone	++++	+	++++
Chloroform	0	+	lysed
Methyl alcohol	++++	+	++
10% Formalin	+++	+++	+++

IV. Discussion.

As shown in Table 1 the staining intensity of the cytoplasm of the liver cells was either reduced markedly or blocked by all the lipid solvents used. This indicates that the compound is either a lipid or a lipid containing complex. Extraction with pyridine was more effective than the others and it should be remembered that Baker[7] used loss of staining after pyridine extraction as proof that phospholipids were stained by his acid hematein technic. Koenig[8] compared the staining of spinal cord following extraction with chloroform-methanol (2–1) with that of unextracted spinal cord. He found that following extraction, the neurokeratin meshwork failed to stain with the Weil stain. He also found that extracted spinal cord proteolipid could be stained with the Weil stain. Proteolipids contain phosphorus and thus by definition are classed with phospholipids. It is possible that the compound in the liver stained by the Weil stain could be further characterized with extraction procedures. The increase in staining of cell membranes after chloroform-methanol, acetone or methyl alcohol extraction is not explainable at present nor is the heavy staining of red cells after acetone extraction.

The staining intensity was increased by methylation. Since this reaction would esterify any of the compounds with a replaceable H+ the possible reaction of the dye as a base is precluded. Likewise any simple amine would be alkylated and thus would become a substituted amine. Similarly the staining intensity was increased by aldehyde blockage with hydroxylamine. The fact that blockage did not occur eliminates the CHO group. The increase is difficult to explain but it is possible that an addition product formed which acted as a substituted amine. The findings with acetylation are complicated. The results shown in Figure 1 are the same for both spinal cord (Berube, Powers and Clark[3]) and liver. However, Bulmer[9] reported that time in the acid staining bath profoundly affected the intensity of staining with phosphotungstic acid hematoxylin following acetylation. A series of slides was placed in the usual acetylation mixture for three hours, rapidly hydrated and stained for 2, 4, 8, 16 and 32 minutes in the full strength Weil stain (four times as concentrated as our routine stain). Control slides were stained in the same solution and for the same length of time and were differentiated identically. The optical density of these slides was measured by the method previously used[2]. Then the density of each acetylated slide was divided by the density of its control. The values found were: 2 min, .87; 4 min, .92; 8 min, 1.04; 16 min, 1.07; 32 min, 1.36. It is evident that the stain was partially blocked initially and that overstaining developed slowly. This is consistent with Bulmer's[9] concept that hydrolysis of the acetylated compounds occurs in the acid staining solutions. The suggestion might be advanced that complete acetylation blocks the stain while after partial hydrolysis some materials may act as a substituted amine. The results of amine blockage and deamination are almost conclusive in themselves in implicating an amine as the functional group to which the dye is attached but explanation of the results of acid extraction are more difficult. Presumably the acid removed some compound which then allowed an amine to react with the dye.

There are, of course, many side reactions with blocking procedures so it is hardly advisable to make dogmatic statements. The total body of the results, however, makes it possible to suggest that the compound which decreases in cold adaptation but increases in poisoning with halogenated hydrocarbons is a phospholipid containing a secondary amine. This secondary amine is the functional group to which the Weil stain attaches in the liver as well as in the spinal cord.

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