from that of *Oryzaephilus*[9] which can do well without leucine, lysine, threonine or phenylalanine but requires cystine and glycine.

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Résumé

On décrit un milieu de composition chimique définie, se prêtant à l'analyse qualitative des acides aminés nécessaires au développement des larves de *Trogoderma*. A cet effet, dix acides aminés sont indispensables: l'arginine, l'histidine, l'isoleucine, la leucine, la lysine, la méthionine, la phénylalanine, la thréonine, la tryptophane et la valine. Le lard et l'acide nucléique sont également nécessaires à l'alimentation de ces larves.

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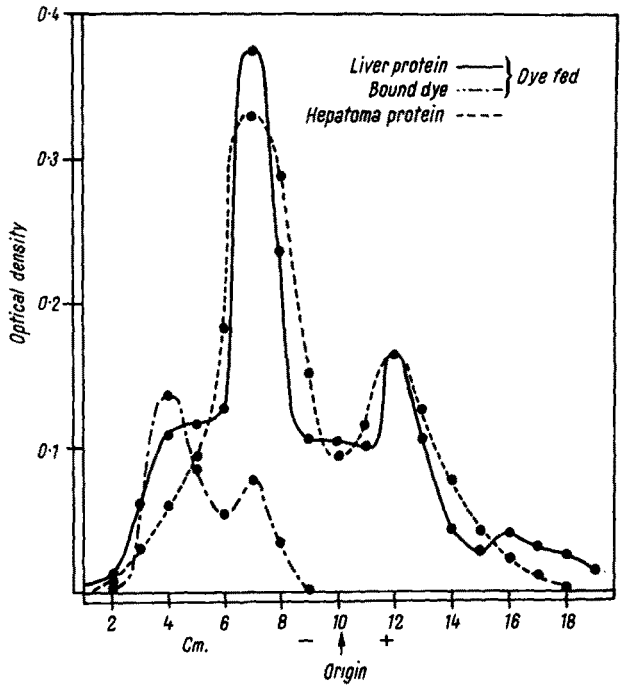
Studies on the Dye-Binding Fraction of Soluble Liver Proteins from Rats Fed Aminoazo Dyes[1]

E. C. MILLER and J. A. MILLER[2] observed, when carcinogenic azo dyes are fed to rats, the dye is bound by covalence to certain proteins in the liver, prior to the appearance of tumors. Two-third to three-fourth of the cytoplasmic bound dye is attached to a particular electrophoretic fraction, which has been called the 'h' proteins by SOROF *et al.*[3]. Since this 'h' fraction is considerably lowered in hepatomas induced with azo dyes[3], the idea was advanced by E. C. MILLER and J. A. MILLER[4] that the 'h' protein may play a role in the carcinogenic process. A brief account is given of various studies on this 'h' fraction, isolated in mg amounts by electrophoresis on starch, and of a comparative study of the electrophoretic patterns of rat liver and hepatoma supernatant fluids.

The Figure shows the typical distribution pattern of the supernatant fluid from the liver of rats fed for 2 weeks 3'-Methyl-*p*-dimethylaminoazobenzene (3'-Me DAB). About two-third of the dye is associated with the first shoulder from the left on the protein pattern, the 'h' fraction. It is interesting, that the electrophoretic patterns of the soluble protein and of the bound dye, obtained with the relatively non-carcinogenic isomer 2-Me DAB, (2 weeks), have shown an overall similarity to those of the carcinogenic 3'-Me DAB (Figure). The supernatant fluid, obtained from rats fed the same semi-synthetic diet, but containing no dye, gave also essentially the same pattern. However, the 'h' fraction and the bound dye were absent in the supernatant fluid of hepatoma induced with 3'-Me DAB (Figure). The isolation of mg amounts of 'h' fraction was carried out in similar conditions, but $2 \times 5 \times 31$ cm blocks were used and 1500 mg samples of lyophilizate; time 21–24 h. After dialysis and lyophilization of the extracts of the corresponding segments, the yield was 5–10 mg 'h' protein per block.



Electrophoretic patterns on starch block of liver and hepatoma supernatant fluid of rats fed 0·06% 3'-Me DAB in a riboflavin low semi-synthetic diet. 150 mg samples of lyophilizate in sucrose were used, containing 20% protein. The parallel, horizontal blocks were $1 \times 2 \times 31$ cm. The electrophoresis was carried out in a 0·02 *M* pH 7·4 barbital buffer, containing also 0·055 KCl/l, at 225 V, with 10–12 mA per block, during 12–16 h. The protein distribution has been followed by a modified Folin reaction and the bound dye pattern by measuring the optical densities at 525 mμ in formic acid, on the extracts of the segments of the blocks.

Amino acid composition. Paper chromatography of the hydrolyzates of both the normal and 3'-Me DAB containing 'h' fractions, by the concurrent use of the methods of MCFARREN[5] and CLAYTON and STRONG[6], has shown no qualitative differences in amino acid composition. The chromatograms showed for both high proportion of leucin, alanine, valine, lysine, and probably arginine. Cystine and cysteine were determined quantitatively after NAKAMURA and BINKLEY[7]. The percentage of tyrosine and tryptophane was measured by U.V. absorption after BEAVEN and HOLIDAY[8]. The following amino acids have been identified: aspartic and glutamic acids; serine; glycine; threonine (traces); alanine; cysteine (normal: 0·18%, dye containing: 0·22%); cystine (normal: 0·21%, dye containing: 0·15%); arginine: lysine; proline; histidine; tryptophan (normal: 0·95%, dye containing: 0·94%); tyrosine (normal: 3·24%, dye containing: 4%); methionine; valine; leucine; phenylalanine. The cystine + cyst-

[1] This investigation was supported by the Institutional Grant 71 from the American Cancer Society and the Grant C-355 of the National Cancer Institute, Public Health Service to Drs. J. A. MILLER and E. C. MILLER, to whom we are greatly indebted.

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[8] G. H. BEAVEN and E. R. HOLIDAY, Adv. Protein Chem. *7*, 319 (1952).

	B_1	B_2	Niac.	Biot.	Folic	B_6	B_{12}
Normal 'h'	7	3·5	63·0	0·4	1·8	7·7	0·4
'High peak' from normal	—	45·1	—	—	—	—	—
'High peak' from 3'-Me DAB fed .	—	12·7	—	—	—	—	—

eine content of the 'high peak' (corresponding to 6–8th cm on the Figure was also determined (normal: 0·13%, dye containing: 0·11%).

Vitamin content. Only trace amount of vitamins have been found in the normal 'h' protein by microbiological assay. The riboflavin content of the 'high peak' was determined by fluorometry after BURCH *et al.*[9]. There is possibly a competition for binding in this segment between 3'-Me DAB and riboflavin, since only about one-fourth of the normal level of vitamin was found in this fraction, when it was isolated from dye containing supernatant fluid. The following Table gives the vitamins in μg/g of protein.

Nucleic Acids. The stepwise analysis of the perchloric extracts of the 'h' fraction has shown the complete absence of purine and pyrimidine bases. Thus, this fraction is devoid of nucleic acids and their acid soluble components, in good agreement with the results of SOROF *et al.*[10].

Molecular weight and bound dye. The molecular weight distribution of the 'h' fraction was studied in an analytical ultracentrifuge[11]. Two, nearly equal fractions have been observed, one rapidly moving (M.W. $\sim$ 45,000), an other slowly moving (M.W. $\sim$ 90,000).

The amount of bound 3'-Me DAB per unit weight of 'h' fraction was determined by colorimetry in 88% formic acid. 8 mg of this protein contained 2·15 γ of bound dye. Thus, assuming for the 'h' fraction an average molecular weight of 67,000 and an even distribution of the dye, only 27·2% of the protein molecules contain bound dye.

A full account of these and related investigations will be given elsewhere.

G. H. WIRTZ[12] and J. C. ARCOS[13]

McArdle Memorial Laboratory, University of Wisconsin, Medical School, Madison, Wis., December 24, 1957.

Résumé

Les auteurs ont étudié le profil électrophorétique du surnageant de foies de rats, nourris aux colorants azoïques cancérogènes. La fraction, dite protéine «h», a été isolée et on a déterminé son poids moléculaire, sa teneur en colorant lié, en vitamines et en acides nucléiques et sa composition en acides aminés.

[9] H. B. BURCH, O. A. BESSEY *et al.*, J. biol. Chem. *175*, 457 (1948); *180*, 755 (1949).

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[11] The molecular weight determinations were carried out by Dr. HAROLD F. DEUTSCH, to whom our thanks are due..

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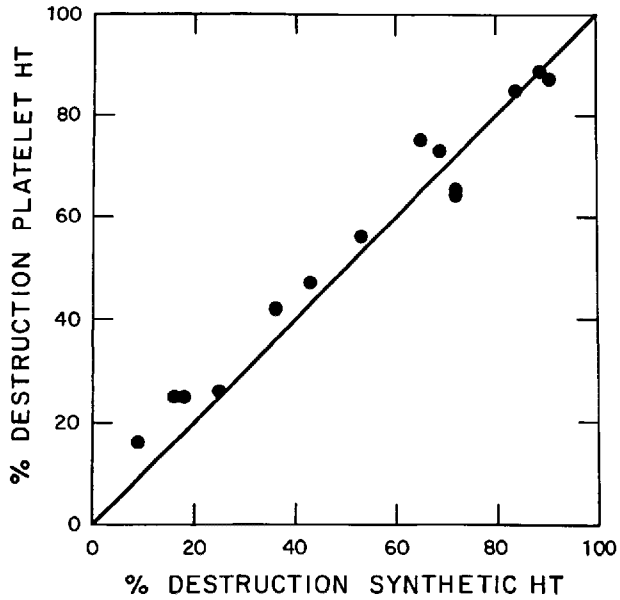
Serotonin Storage Mechanism and its Interaction with Reserpine

Serotonin (5-hydroxytryptamine, HT), a possible neurohumoral agent, is said to be 'bound' since tissue cells contain the amine in a form not immediately available to exert a biologic action. In studying HT storage, HUMPHREY and TOH[1] were the first to report that platelets can take up HT against a concentration gradient. The mechanism for HT uptake is not known; it could involve complexing of HT with an intracellular constituent or its passage across the cell boundary by active transfer. The present studies are concerned with the nature of the concentrating mechanism and how it is affected by reserpine.



Action of Monoamine Oxidase on Serotonin (HT) from Lysed Platelets. 0·1 to 2 ml of monoamine oxidase preparation was incubated with suspensions of disrupted platelets and with solutions of synthetic HT at pH 7·4 in air for 15 or 30 min at 37°C. Residual HT was measured as described previously[4]. Fraction of HT destroyed in lysed platelets is plotted against that destroyed in solutions of synthetic HT. The line represents the theoretical slope if the fraction of HT destroyed in lysed suspensions and in synthetic HT solutions were identical.

Evidence for a HT complex was sought in rabbit platelets which contain HT at a concentration thousands of times that in plasma. Platelets were isolated[2] at 4°C, washed twice with cold 0·1 *M* phosphate buffer, pH 7·4, suspended in 1 ml of buffer, lysed with 9 ml ice-cold water, and the suspension was subjected to ultrafiltration[3]. HT

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[2] G. H. L. DILLARD, G. BRECHER, and E. P. CRONKITE, Proc. Soc. exp. Biol. Med., N. Y. *78*, 796 (1951).

[3] P. P. REHBERG, Acta physiol. scand. *5*, 305 (1943).