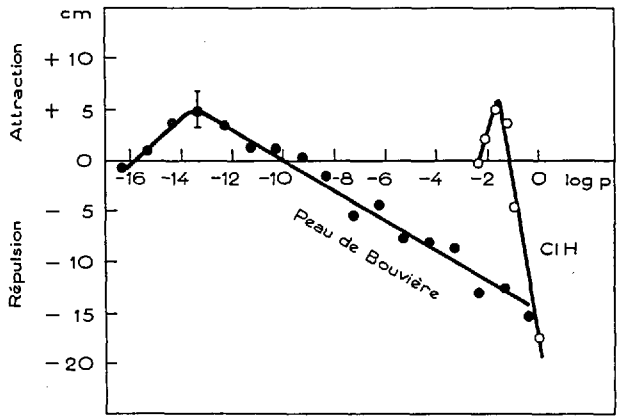
Sur la Figure sont portés les résultats obtenus avec l'acide chlorhydrique et avec la peau de bouvières. En abscisses les logarithmes du poids du stimulus employé contenu dans le cm³ de solution imbibant le papier-filtre et en ordonnées les répulsions (ordonnées négatives) ou les attractions (ordonnées +) obtenues.



Chimiotropisme des bouvières. Attractions et répulsions en fonction du logarithme du poids p du stimulus employé: Peau de bouvière, ClH.

Dans les deux cas on voit qu'il y a répulsion pour les fortes concentrations du stimulus et attraction pour des concentrations plus faibles. En partant des concentrations liminaires et en faisant croître la concentration, on voit que la courbe passe par un maximum d'attraction.

Pour nous faire une idée de la précision des mesures nous avons marqué sur la Figure par un trait vertical la dispersion des valeurs mesurées en répétant l'expérience 10 fois avec un lot de 5 bouvières chaque fois pour log $p = -13{,}4$ (maximum de l'attraction).

La moyenne des 10 mesures x_1 a donné une attraction moyenne $\bar{x} = +4{,}82$ cm. Le calcul de l'erreur type sur la moyenne $(\sigma_{\bar{x}_1})$ a donné

$$\sigma_{\bar{x}_1} = \pm \sqrt{\frac{\Sigma (x_1 - \bar{x})^2}{n\,(n-1)}} = \pm \sqrt{\frac{8{,}4}{10{,}9}} = \pm 0{,}306 \text{ cm.}$$

Pour les 10 mesures témoins nous avons trouvé une position moyenne du C.G. décalée de $-0{,}11$ cm du milieu de la cuve avec erreur type sur la moyenne $\sigma_{\bar{x}_2} = \pm 0{,}155$ cm, écart non significatif.

La valeur moyenne de l'attraction réelle est égale à $4{,}82 + 0{,}11 = +4{,}93$ avec une erreur de

$$\sqrt{(\sigma_{\bar{x}_1})^2 + (\sigma_{\bar{x}_2})^2} = 0{,}343.$$

La valeur du test «t» de Student-Fisher (18 degrés de liberté) est alors $4{,}93/0{,}343 = 14{,}4$. L'attraction mesurée par la différence des moyennes est donc significative au seuil de 0,001.

Cet exemple montre que le dispositif décrit[1] est très précis et se prête bien à la mesure d'attractions et de répulsions chimiotropiques. On peut s'en servir aussi pour l'étude du phototropisme et aussi pour l'étude de la répulsion ou de l'attraction causée par d'autres facteurs physiques.

E. HEINTZ

Laboratoire de Psychologie Animale, Institut de Zoologie et de Biologie Générale de l'Université de Strasbourg, le 20 décembre 1957.

Zusammenfassung

Chemotaktische Messungen an Bitterlingen (*Rhodeus amarus* B.), die dem Einfluss verschiedener Mengen von Fischhaut und von ClH ausgesetzt waren, werden mit Angabe der Messgenauigkeit beschrieben.

PRO EXPERIMENTIS

Losses of Nucleic Acid Derivatives from Fixed Tissues during Flattening of Paraffin Sections on Water[1]

It is generally recognized that losses of proteins and carbohydrates may occur, if paraffin sections of freeze-dried tissues are flattened on water surfaces prior to the attachment to slides[2]. However, in sections from fixed tissues, either fresh or freeze-dried, mainly the carbohydrates should be affected; the possible release of protein and nucleic acid derivatives has largely been neglected, and no information seems available about losses actually observed. The present note demonstrates such losses from differently fixed and embedded rat pancreas.

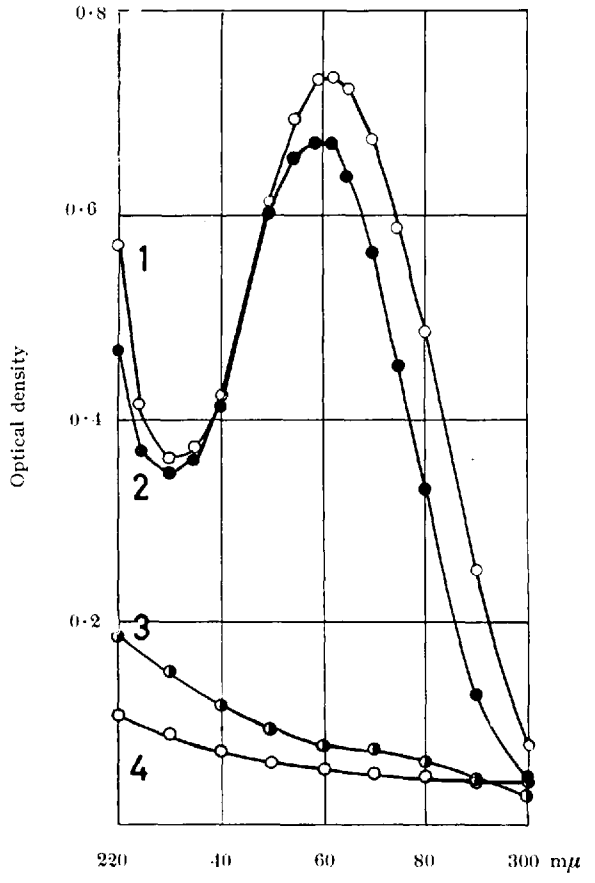


Fig. 1

[1] Aided by a grant from The Royal Physiographic Society, Lund.

[2] A. G. E. PEARSE, *Histochemistry* (Churchill, London 1954) (with further references).

Experiments and Results. Small pieces of pancreas from rats were fixed in Carnoy's fluid for 1·5 h, either fresh or following freeze-drying, or, fresh, for 24 h in LILLIE's buffered formaldehyde solution[3]. Dehydration followed in alcohol. Details of the procedures used were given

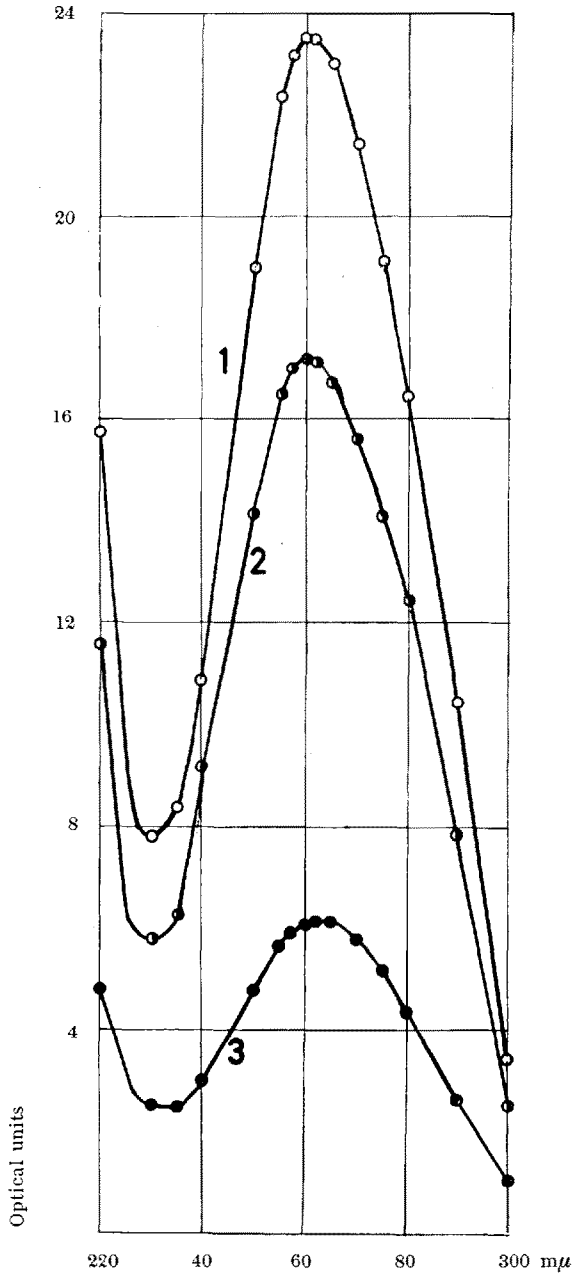


Fig. 2

recently[4]. The following alternative embedding techniques were employed: (1) Benzene for twice 20 min, molten paraffin for 2 h. (2) Methyl benzoate for 24 h, benzene and paraffin as above. (3) Petroleum ether for three times 2 h, embedding in paraffin *in vacuo*. The paraffin (M.P. 52°C) was kept at 56° ± 0·1°C, and solidified at ice-water temperature.

[3] R. D. LILLIE, *Histopathologic Technic* (Blakiston, Philadelphia 1948).

[4] S. LAGERSTEDT, Z. Zellforsch. *45*, 472 (1957). – N. JONSSON and S. LAGERSTEDT, Exper. *13*, 321 (1957).

10 μ sections were distributed between glass-stoppered centrifuge tubes, each tube given the consecutive section. Without de-paraffination, the material was extracted with glass-redistilled water at 37° ± 0·01°C for 5, 10, 20 or 40 min, and the ultraviolet absorption measured in a Beckman Model DU spectrophotometer, using 10 mm cells. As shown in Figure 1, even 5 min extraction of freeze-dried (Curve 1) or fresh (Curve 2) Carnoy-fixed tissues released substances selectively absorbing around 260 mμ. Formaldehyde fixation largely prevented such losses (Curve 3). Curve 4 shows the non-specific absorption caused by the paraffin used for embedding. Increase of extraction time, or variation of embedding techniques did not influence these general results.

The water extracts were evaporated to dryness *in vacuo* at + 45°C, hydrolyzed with *N* HCl at 100°C for 1 h, and spots placed on Whatman No. 1 chromatographic paper. Development in *iso*-propanol/HCl/H_2O: 97/25/28, with a hydrolysate of yeast nucleic acid as reference, yielded four spots on the ultraviolet print: guanine, adenine, cytidylic and uridylic acid. They were further characterized through their absorption spectra. Ninhydrine spraying revealed some admixture of protein degradation products.

In about 30 experiments the losses observed were quantitatively determined, as shown in Figure 2. Curve 3 derives from a 10 min water extract, made up to normal with perchloric acid; Curve 2 from an extract of the same sections after deparaffination with normal perchloric acid at room temperature for 2 h[4]; Curve 1 from a similar perchloric acid extract of a comparable sample of deparaffinized sections from the same block. The sum of the optical units released at 260 mμ for Curves 2 and 3 fits the value for Curve 1 fairly well. This total loss of about 25% of the initial amount was reached within 5 min and not significantly increased through 40 min extraction.

Extraction of paraffin sections with 70% ethanol, sometimes used as a substitute for water in flattening of sections, also caused losses of nucleotide material. Absolute ethanol, in itself, showed no extractive power, of course.

The above findings were verified, at least qualitatively, under the conditions of ordinary histotechniques. Paraffin sections floated on water of different temperatures were observed through binoculars, and the time necessary for complete flattening of the paraffin noted with a stopwatch. At 50°C the paraffin disintegrated quickly, leaving stretched sections; at 37° and 40° the paraffin flattened, but the sections still showed wrinkles; at 43°, 45° and 48° both paraffin and sections stretched satisfactorily. The results are summarized in Figure 3 for fresh Carnoy-fixed tissue, embedded *via* benzene. 16 sections of this material, 10μ thick and containing about 25 mm² of tissue each, were floated for 12 s on 2 ml of water at 45°C. Ultraviolet curves from the water corresponded exactly in type to Curves 1 and 2 in Figure 1. Quantitative estimation, using a slight modification of the method given above, demonstrated losses of about 6%.

Conclusions. Obviously, proteins and nucleic acid derivatives are not protected by the paraffin in paraffin sections from extraction by water during the histotechnical flattening procedure. It seems probable, in the material tested, that maximum extraction is accomplished rather quickly (25% after 5 or 40 min), and that flattening performed even under optimal conditions (12 s at 45°C) will not prevent from such losses. Thus, in critical histochemical work, the stretching on water surfaces must be avoided, or, at least, the effect carefully controlled for each tissue and fixative used. Since the formaldehyde

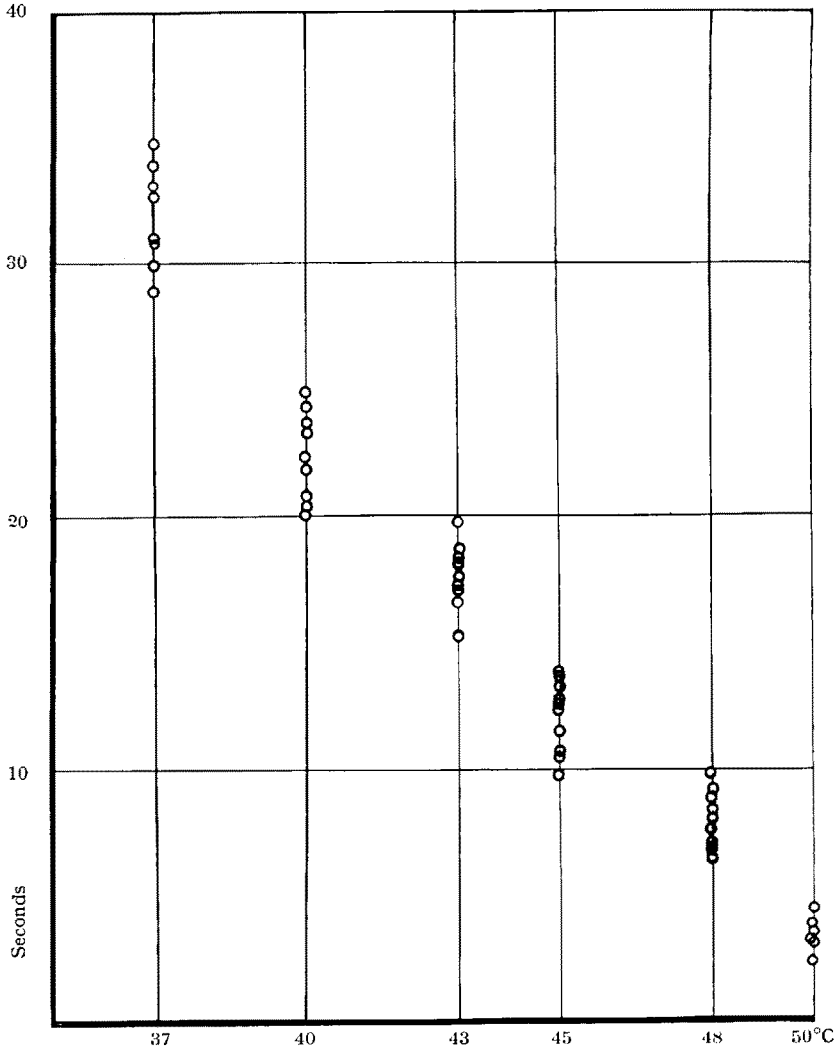


Fig. 3

fixative itself extracts nucleotide material[4], this method seems largely unsuitable. Consequently, the preparation of microtome sections of fresh-frozen tissue[5] in recent modifications[6], or mounting of the sections on slides without using water, has to be adopted.

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Department of Histology, University of Lund (Sweden), November 5, 1957.

Zusammenfassung

Aus fixiertem Gewebe bzw. Paraffinschnitten, die mit normaler Technik auf Wasser ausgebreitet werden, gelingt es, Nukleotide und Eiweissderivate zu extrahieren. Quantitative Daten werden mitgeteilt, und es wird deren Bedeutung für die Histochemie diskutiert.

[5] K. Linderstrøm-Lang and K. R. Mogensen, C. R. trav. lab. Carlsberg Sér. Chim. *23*, 27 (1938).

[6] B. W. Grunbaum, J. R. Geary, Jr., and D. Glick, J. Histochem. Cytochem. *4*, 555 (1956). – W. Thornburg and P. E. Mengers, J. Histochem. Cytochem. *5*, 47 (1957).

PRO EXPERIMENTIS

Adaptation of the Methyl Orange Method for the Determination of Reserpine*

The method proposed in the present paper represents an adaptation of a general reaction for organic bases developed by Brodie and Udenfriend[1], and has the advantages over previous methods[2] of being simple, rapid, and applicable to both pharmaceutical preparations and biological materials. Reserpine is extracted from slightly acidic solutions (pH adjusted to between 4–6 with 0·1 N HCl) with five times as much ethylene dichloride (the solvent phase is washed with an equal volume of 0·1 N sodium hydroxide), and then reacted

* A grant for this study, and the reserpine used in these experiments, were generously supplied by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

[1] B. B. Brodie and S. Udenfriend, J. biol. Chem. *158*, 705 (1945).

[2] S. M. Hess, P. A. Shore, and B. B. Brodie, J. Pharmacol. *118*, 84 (1956). – A. J. Glazko, W. A. Dill, L. M. Wolf, and A. J. Kazenko, Pharmacol. *118*, 377 (1957). – R. B. Poet and J. M. Kelley, 126th National Meeting of the A.C.S., Division of Biological Chemistry, New York (September 1954).