Während dieser Zeit ist die 5-OT-Aufnahmefähigkeit der Plättchen vermindert. Der Oxytryptamingehalt der Plättchen erreicht seinen Ausgangswert erst ungefähr 3 Wochen nach der Reserpingabe.

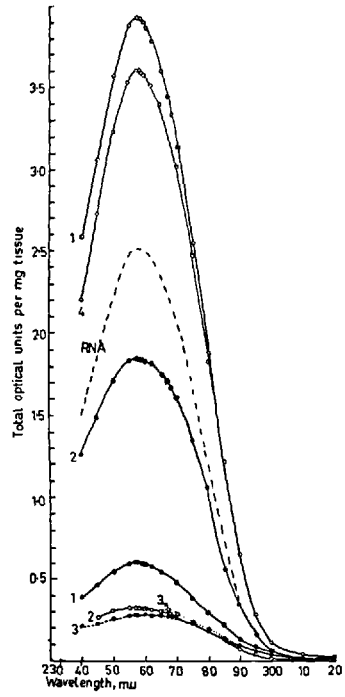
Die Ansicht, dass die Plättchen freies 5-Oxytryptamin aus dem Kreislauf aufnehmen, wäre mit diesem Ergebnis zu vereinbaren. Die Dauer des Effektes könnte bedeuten, dass Reserpin entweder auf Plättchenvorstadien im Knochenmark wirkt oder aber dass die Lebensdauer der Plättchen in Wirklichkeit länger ist, als neuerdings angenommen wird.

The Release of Ribonucleic Acid from Carnoy Fixed Sections During Incubation in McIlvaine's Buffer

Formaldehyde solutions have been preferred as fixatives for tissues prior to the histochemical ribonuclease (RNase) test, to the disadvantage of CARNOY's solution[1]. The main objection towards the latter is that sections incubated in a buffer without the addition of RNase, lose their staining properties for ribonucleic acids (RNA), just as in the presence of the enzyme. Thus, it is not possible, in the absence of a 'blank', to distinguish the effect of an 'un-specific' degradation of RNA and, perhaps, also of other substances due to the action of the buffer, from that of the specific enzymic break-down of RNA molecules. In contrast to this, in sections from formaldehydefixed tissues, RNA-containing structures still bind basic dyes after treatment with the enzyme-free buffer solution.

However, good solubility of RNA in Carnoy-fixed sections might indicate a better preservation of the native RNA molecules than after formaldehyde fixation. In that case, the ease with RNA is released from the fixed tissue will not affect the specificity of the test. Thus the main question seems to be whether substances other than RNA degradation products can be detected in the buffer extracts from Carnoy-fixed sections, and to what extend the release of RNA will be complete, as compared to digestion in the presence of RNase. The present communication reports the results from a comparative analysis of the ultraviolet absorbing substances liberated from CARNOY and formaldehyde-fixed tissues in McILVAINE's buffer, and in the same buffer containing RNase.

Experimental.—Fresh and frozen-dried pieces of rat pancreas were fixed in CARNOY's solution (ethanol : chloroform : acetic-acid – 6 : 3 : 1) for 1·5 h, or fresh pieces in buffered (pH 7·0) 10% formaldehyde solution[2] for 24 h at room temperature. They were transferred *via* the appropriate intermedia to paraffin (m.p. 55°C), as described elsewhere in detail[3]. From each paraffin block, 7 μ thick sections were cut, and distributed equally between centrifuge tubes in such a way that all the tubes contained comparable lots of the material. Some sections were mounted on slides for histochemical control. After deparaffination in petroleum ether, the sectioned material was spun down in a centrifuge, further washed with ether, and allowed to dry at room temperature. The samples were weighed, and each tube contained approximately 10 mg dry material. Some tubes with sections from fresh Carnoy-fixed and frozen-dried Carnoy-fixed tissue were submitted to secondary fixation in the buffered formaldehyde solution for 24 h. They were then freed from the formaldehyde through dialysis in cellophane sacs against distilled water in the cold room for 24 h. Thus the experiments included samples of sections which had been fixed accordingly: (1) Fresh Carnoy fixation. (2) Frozen-dried Carnoy-fixed. (3) Fresh formaldehyde fixation. (4) Frozen-dried Carnoy-fixed and then formaldehyde-fixed.



McILVAINE's buffer was used at pH 7·0, in the digestion experiments containing 1 mg crystalline ribonuclease (WORTHINGTON) per milliliter. Incubations were performed at 37°C for 2 h, and with mechanical stirring. The sections were spun down between each treatment, and the supernatants collected. 5 ml samples were used throughout. To test the completeness of the RNAS digestion, the sections were extracted with *N* perchloric acid for further 3 h at room temperature, as appropriate for pancreatic tissues[3]. Thus, in the actual set of experiments, each of the four types of preparation was treated according to the following scheme: (1) McILVAINE's buffer for 2 h. (2) RNase containing buffer for 2 h. (3) *N* perchloric acid for 3 h. All experiments were performed in double. In addition, direct digestion with RNase containing buffer for two times 2 h was performed on all types of preparation.

The supernatants were investigated in the Beckman Modell DU quartz spectrophotometer in the ultraviolet range, using 10 mm cells, against the corresponding solutions as a blank. Making use of the known volumes and weights of the samples, the absorptions were calculated as total optical units per milligram tissue, and are thus given in the graph.

The results are presented in the Figure. The curves with the un-filled circles represent the results from incubation of frozen-dried Carnoy-fixed sections. Curve 1

[1] R. E. STOWELL and A. ZORZOLI, Stain Techn. *22*, 51 (1947). – B. P. KAUFMANN, H. GAY, and M. R. MCDONALD, Cold Spring Harbor Symp. quant. Biol. *14*, 85 (1950). – A. G. E. PEARSE, *Histochemistry* (Churchill, London 1954). – N. B. KURNICK, Int. Rev. Cytol. *4*, 221 (1955).

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

[3] S. LAGERSTEDT, Z. Zellforsch. (in press.).

shows the absorption of McILVAIN's buffer extract, and curve 2 that of the following RNase digest. Obviously, only very small amounts of RNA were left in the sections after extraction with the buffer solution. The curve from the *N* perchloric acid extract (curve 3) coincides extremely well with that from the RNase digest, indicating the completeness of the digestion. Curve 1 shows close similarity to a curve from a primary digest of sections with RNase under corresponding conditions (curve 4). The quotient *E* 257 mμ/*E* 275 mμ of about 1·5 for both curves, and the coinciding absorption maxima at 257 mμ, are in agreement with the curve from a digest of protein-free yeast nucleic acid in the same system (the dotted reference curve), indicating the absence of appreciable amounts of proteins.

The curves with the filled circles demonstrate the results with frozen-dried Carnoy-fixed and then formaldehyde-fixed sections. McILVAINE's buffer alone (curve 1) will remove about 20% of the total absorption at 257 mμ, but the main bulk of the absorbing substances is liberated in the following digestion with RNase (curve 2). Curve 3 (*N* perchloric acid extract) shows the completeness of the RNase digestion.

The same results are obtained using fresh Carnoy-fixed, fresh formaldehyde-fixed, or fresh Carnoy- and then formaldehyde-fixed tissues. The quantitative discrepancy observable between the total optical units liberated from the formaldehyde and the Carnoy-fixed material, as obvious in the Figure, has been shown to be caused by the extraction of acid soluble nucleotides by the formaldehyde fixative, and has been analyzed elsewhere[3].

For the identification of the absorbing substances in the McIlvaine-buffer-extract, use has been made of paper chromatographic analyses. The extracts were mixed with the equal volume 2 *N* HCl, and hydrolyzed for 1 h at 100°C. The products were adsorbed on charcoal, washed with water on the filter, and eluted with 50% ethanol, containing 2% NH_3. After concentration to a small volume *in vacuo*, spots were placed on Whatman No. 1 filter paper, and developed with the *iso*-propanol/HCl/H_2O solvent (97:25:28). The ultraviolet-prints, with a sample of protein-free yeast nucleic acid (SCHWARTZ, purified according to VISCHER[4]), dissolved in McILVAINE's buffer, and treated in the same way as the test samples, as a reference, showed the presence of guanine, adenine, cytidylic acid and uridylic acid in the hydrolysate from the buffer extract of Carnoy-fixed sections. Much fainter (especially for guanine and adenine), spots indicated their presence in the extract from the formaldehydetreated sections. The spots were cut out and identified spectrophotometrically, dissolved in 0·1 *N* HCl. No traces of thymine could be detected.

Discussion.—The findings show that Carnoy-fixed sections in McILVAINE's buffer will release all nucleotides digestible with RNase and/or extractable with perchloric acid. No admixture of proteins could be quoted. From these points of view, no objections could be raised against the use of Carnoy-fixed tissues for the histochemical RNase test. The completeness of the extraction by the buffer solution might presumingly be due to the formation of dialyzable degradation products from the RNA. Such a degradation could be accomplished in at least three ways: (1) RNA is hydrolyzed through the action of the acid fixative; this would not be in accordance with the experiences from nerve cells[5]. (2) Electrolytes have been shown to degrade un-fixed (from the histological point of view) RNA[6]. (3) RNase-activity might be present in the sections, and, after addition of the buffer, initiate conditions equivalent to those during the histochemical RNase digestion. Further experiments must be made to decide between these possibilities.

The assumption that formaldehyde-fixed sections do not lose RNA due to the action of the buffer seems doubtful, since about 20% of the total RNase-digestible material has been found in the buffer extract. It is very probable that this fraction is derived from RNA, since formaldehyde solutions will extract the acid soluble nucleotides[3], thus leaving only RNA as a source for the absorbing substances observed. Prolonged extraction will not increase the yield of nucleotides. Since the RNA in the sections is fully accessible to RNase digestion, none of the original RNase activity seems to be left after formaldehyde-fixation.

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Zusammenfassung

Aus den Befunden geht hervor, dass mit dem Puffergemisch von McILVAINE Ribonukleinsäure und säurelöslichen Nukleotide auch bei Abwesenheit von Ribonuklease aus Carnoy-fixiertem Gewebe vollständig herausgelöst werden, und zwar wahrscheinlich ohne Beimischung anderer für die Ribonukleasereaktion nichtspezifischer Substanzen. Nach Fixierung in Formaldehyd geht nur $^1/_5$ der ultraviolettabsorbierenden Substanzen in das Puffergemisch über.

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17-Hydroxycorticosteroids in Human Milk

The aim of the present investigation has been to determine the level of 17-hydroxycorticosteroids in human milk, and also the ability of cortisone or hydrocortisone administration to elevate this level. The elucidation of this point is of pediatric significance in cases when the illness of the nursing mother (e.g., rheumatic fever or rheumatoid arthritis[1]) necessitates the administration of glucocorticoids.

The determinations have been extended to refer also to the glucuronic acid conjugated forms of 17-hydroxycorticosteroids.

Material. The specimens of human milk were collected from eight healthy mothers on the second or third day after delivery, at the following times: (1) 8 a.m. (2) 2 p.m. (3) 8 a.m. the next day. Immediately after the taking of the first specimen a single intramuscular injection of 100 mg cortisone acetate was given to four mothers. The remaining group of four mothers received a corresponding injection of 100 mg hydrocortisone acetate.

Method. The fresh samples of human milk were centrifuged (r.p.m. 8500, temp. + 5°C). Aliquots of 5 cm^3 were taken from the clear bottom layer for the estimation of the 17-hydroxycorticosteroids. The free

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