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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[5] J.-E. EDSTRÖM, Biochem. biophys. Acta *12*, 361 (1953).
[6] J. P. GREENSTEIN, C. E. CARTER, and H. W. CHALKLEY, Cold Spring Harbor Symp. quant. Biol. *12*, 64 (1947).

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

[1] M. OKA, Ann. Rheum. Dis. *12*, 227 (1953).

17-hydroxycorticosteroids were determined by following the method of SILBER and PORTER[2] as modified by PETERSON[3]. After the extraction, the supernatant was saved for the determination of the conjugated forms. ½ cm³ of 2 *M* phosphate buffer pH 6·2 and 400 units of bacterial β-glucuronidase (*Σ*) were added. The re-extraction was performed after 48-h incubation at + 37°C.

Recovery of added hydrocortisone from human milk

Amount of added hydrocortisone (γ/5 cm³)	Recovery %
½	83
½	87
1	87
1	92
2	89
2	97

The suitability of the method for the estimation of 17-hydroxycorticosteroids from milk was checked by a recovery study of added hydrocortisone (Table).

Results. The values of the *free* 17-hydroxycorticosteroids before the administration of cortisone or hydrocortisone acetate ranged from 0 to 2·6 γ% in the eight cases studied. These concentrations are so low that the accuracy of the method used is no longer reliable. The single intramuscular injection of 100 mg hydrocortisone acetate produced a very slight elevation in the PORTER-SILBER reacting material. 6 h after the injection the values ranged from 2·2 to 4·7 γ%, and after 24 h from 1·5 to 3·0 γ%. The increase in the PORTER-SILBER reacting material after the injection of cortisone acetate was still smaller.

The level of the *conjugated* 17-hydroxycorticosteroids in the eight human milks studied ranged from 0·2 to 3·4 γ%. The single intramuscular injection of 100 mg cortisone or hydrocortisone acetate produced a slight elevation in the values of the conjugated 17-hydrocorticosteroids only in three instances in the samples drawn 6 h after the injection. All the values were near zero 24 h after the injections.

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Kivelä Hospital and The Womens Clinic of the University of Helsinki, July 8, 1956.

Zusammenfassung

Der Gehalt an freiem und gebundenem 17-Hydroxycorticosteroid wurde an 8 Muttermilchentnahmen nach einer Modifikation der Methode von SILBER und PORTER geprüft. Der erhaltene Wert war nichtsignifikant niedrig. Auf einmalige intramuskuläre Injektion von 100 mg Cortison oder Hydrocortison erfolgte kein signifikanter Anstieg des Steroidwertes.

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[3] R. E. PETERSON, J. B. WYNGAARDEN, S. L. GUERRA, B. B. BRODIE, and J. J. BUNIM, J. clin. Invest. *34*, 1779 (1955).

The Effect of LSD-25 upon the Cerebral Blood Flow and EEG in Cats

The object of the present study was to determine whether LSD-25 (*d*-lysergic acid diethylamide[1]) has any effects upon the cerebral blood flow, the EEG and the blood pressure in anaesthetized and non-anaesthetized (*encéphale isolé*) cats. In recent experimental studies[2], a sympathomimetic action of LSD-25 in man and experimental animals has been reported. PURPURA[3] has analyzed the effects of LSD-25 upon cortical electrical activity. Recently, SOKOLOFF *et al.*[4] found no changes from LSD-25 in cerebral blood flow, vascular resistance, oxygen consumption, glucose utilization or respiratory quotient in man (normals and schizophrenics). The present experiments have been performed with the method of INGVAR and SÖDERBERG[5] which provides a continuous registration of the cerebral blood flow with a high degree of accuracy.

In cats under pentothal or of the *encéphale isolé*-type, the venous outflow from the cannulated superior sagittal sinus was continuously measured with an electrical drop counter. Anastomoses between diploic veins and the sinus were interrupted by a longitudinal craniotomy. The bony defect was filled with dental acrylate cement. EEG was recorded from rostral and parietal parts of the cortex.

Injected intravenously, LSD-25 in doses of up to 10 μg/kg had no effects upon the functions studied. In larger doses up to 100 μg/kg, however, a small transient cerebral vasoconstriction was observed which coincided with a transient 'activation' of the EEG. Sometimes a small cutaneous vasoconstriction was also noted[6].

Injections into the carotid artery of LSD-25 were also carried out in doses of up to 50 μg/kg. It was then observed that the EEG effects were very pronounced in the homolateral hemisphere. Figure 1 shows a successful example in which 100 μg of LSD-25 were injected into the right carotid artery (*encéphale isolé*-preparation). There was an immediate transient effect of the 'activation' type in both leads from the right hemisphere which, however, was soon followed by a general reduction of the amplitude, an increase in the numbers of spindles and – bilaterally – a generalized decrease in frequency. In about 4 min, the EEG changes had almost disappeared, but a right-sided reduction of the amplitude was still retained.

The flow record shows an immediate vasodilatation followed by a long period during which the flow was practically unchanged in spite of the fact that the blood pressure gradually increased about 30 mm Hg in 5 min. This is interpreted as due to a vasoconstriction in the cerebral vessels. A long-lasting increase of the cerebral vascular resistance was, in fact, the most characteristic effect of LSD-25 seen in the experiments.

In Figure 2 (*encéphale isolé*) another example is given of the effects of 100 μg LSD-25 into the left carotid

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