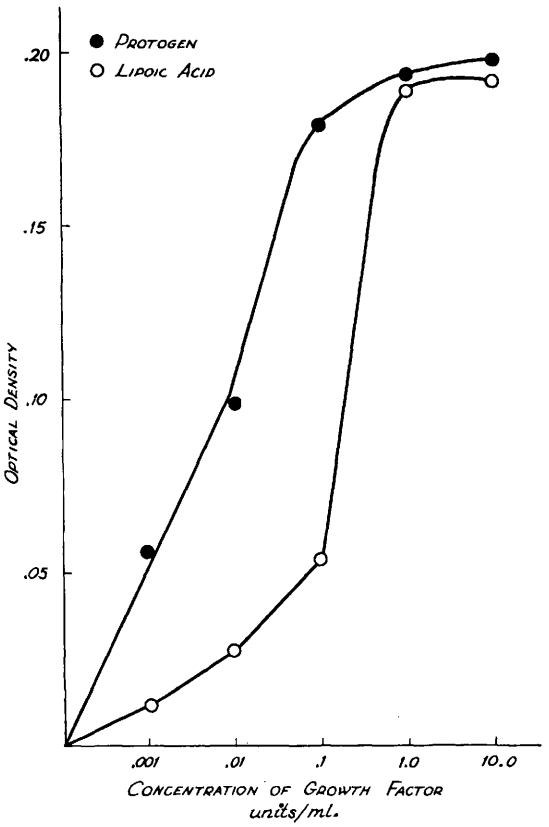

FIG. 1. Growth of *Tetrahymena geleii* S in response to various concentrations of protogen and of lipoic acid.

equal to that obtained in the presence of protogen. Since crystalline samples of neither protogen nor lipoic acid are available at present for investigation, it is of course not possible to designate one factor as more active than the other.

It has been suggested that lipoic acid may be a new B vitamin. It has been shown that there occur a variety of active forms of this factor in nature(6,7); this had been previously suggested by Snell and Broquist(2). Thus lipoic acid appears to resemble such vitamins as pyridoxine and niacin which also occur in nature in a variety of chemical forms. In fact, since lipoic acid replaces protogen, it appears that protogen may actually be either lipoic acid or one of its chemical derivatives.

Summary. Lipoic acid is capable of completely replacing protogen in the growth of *Tetrahymena geleii.*

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Use of Perchloric Acid for Nucleic Acid Histochemistry in Mammalian Nerve and Liver Cells.* (19307)

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Following the report of Ogur and Rosen(1) on the differential extraction of pentose nucleic (PNA) and desoxypentose nucleic acid (DNA) from plant and animal tissues with perchloric acid, there have been several attempts to apply this procedure to the histochemical demonstration of nucleic acid. Ogur and Rosen's procedure has been employed for this purpose on plant tissue(2), a proto-

* Partial accounts were presented at the Annual Meetings of the Histochemical Society March 1950, and 1951.

zoan organism(3), a bacterial species(4), and several mammalian species(5,6). The purpose of this paper is to present qualitative and quantitative data on the use of the perchloric acid extraction procedure in mammalian nervous tissue and liver.

Methods. The brain and spinal cord of cat, dog, rat and guinea pig and the liver of cat and dog, all fixed by vascular perfusion with a solution containing 10% formalin, 2.4% gum acacia and 0.9% sodium chloride(7), were employed in this study. Paraffin sections, 10 μ thick, were carried down to water and placed in 10% perchloric acid. Extraction was carried out at 4°C, 20°C and 37°C for varying periods of time to determine the minimum time necessary for the removal of PNA. Control sections were incubated in water at the same temperature and otherwise treated in an identical manner as the experimental sections. The affinity of nucleic acid for basic aniline dyes was employed to reveal PNA and DNA. Sections were stained with thionin buffered at pH 3.4(8). The Feulgen reaction, carried out according to the recommendations of Stowell(9), was used to reveal DNA. Quantitative determinations of a relative nature were made on the intensity of coloration of glial nuclei and liver cell nuclei with a direct photometric technic employing apparatus modified from that of Pollister and Moses (10). Green light provided by a Wratten filter No. 58 and a tungsten filament light source stabilized with a voltage regulator was used for illumination of the tissue sections. The Millon reaction, modified from Bensley's procedure(11) by carrying the procedure out at 56°C for 1½ hours, was used for the study of nerve cell protein. Photometric measurements of tyrosine mercurial were made at 3650Å by using a G. E. CH_4 lamp and a Corning filter No. 5840(12). The effect of perchloric acid treatment on cell structure was observed in unstained sections with the phase contrast microscope. Sections stained with the acid dyes, eosin and fast green, were also examined for structural alterations. The effect of the perchloric acid extraction procedure on non-specific light losses and specific absorption by tissue constituents other than PNA near the nucleic acid absorption maximum of 2600Å was studied with a direct photometric technic. For illumination a mercury resonance lamp (Hanovia) was used in conjunction with a quartz filter cell containing a solution of nickel sulfate and cobalt sulfate(13) to provide 2537Å radiation. The optical parts of the microscope consisted of a fused quartz condenser, a 50 X reflecting objective of N.A. .54 (American Optical Co.), and a 10 X quartz ocular. Tissue sections mounted in glycerin on quartz slides with quartz coverslips were focussed in visible light and were then ready for photometric measurements or photography in ultraviolet light without refocussing, because the reflecting objective is achromatic. Transverse sections, 10 μ thick, of cat skeletal muscle and spinal cord fixed as above were measured photometrically. The muscle fibres were found to have no cytoplasmic basophilia and were assumed to contain no PNA. These sections were photometered and photographed and the measurements repeated on the same sections after extraction in 10% perchloric acid for 15 minutes at 37°C. The sections of spinal cord were photometered untreated, then incubated in protease-free ribonuclease (0.1 mg/cc of McIlvain's buffer, pH 7.0 at 37°C for 3 hours) for the removal of PNA, photometered again, extracted with perchloric acid as for the skeletal muscle, and photometric measurements carried out the third time on the same section.

Results. Extraction of PNA. The procedure of Ogur and Rosen(1), namely, 18 hour extraction with 10% perchloric acid at 4°C, removed all cytoplasmic and nucleolar basophilia, which depends upon the presence of PNA, from liver cells but proved inadequate in achieving this goal in all the types of nerve cells examined. Even after 93 hours of extraction, considerable amounts of cytoplasmic and nucleolar basophilia remained in neurons. Higher temperatures were there-

TABLE I. Effect of Temperature on Time Required for Extraction of Cytoplasmic and Nucleolar Basophilia with 10% Perchloric Acid.

Temp., °C	Nerve cells	Liver cells
4	Incomplete at 93 hr	18 hr
20	12	3½
25	2	—
37	¼	<1/12

TABLE II. Effect of Perchloric Acid Extraction of Dog Liver and Cat Spinal Cord on Intensity of Feulgen Staining (Mean ± St. Dev. N = 10).

Cell nuclei	Condition of extraction °C	Time, hr	Extinction coef. control	Extinction coef. experimental	Change
Liver	4	18	.11 ± .02	.12 ± .02	0
		93	.15 ± .02	.15 ± .04	0
Astrocytes	20	16	.15 ± .01	.16 ± .01	0
		22	.13 ± .02	.05 ± .03	61.5% reduction Prob. <.01
	37	¼	.30 ± .02*	.29 ± .03*	0
		⅓	.18 ± .01	.15 ± .03	17.5% reduction Prob. <.05

* Central core of nucleus 2 in diam. measured as compared with 3.3 μ diam. core of nuclei for the other measurements.

fore employed for the extraction of nervous tissue. The results of this phase of the study are summarized in Table I. Sections from several blocks of cat spinal cord and brain that had been hardened in 10% formalin for almost 3 years exhibited a marked refractoriness to perchloric acid extraction, considerable cytoplasmic and nucleolar basophilia remaining even after treatment for 1 hour at 37°C. This tissue also proved to be markedly resistant to ribonuclease digestion. It is apparent from Table I that liver cells and nerve cells from the same animal differ consistently with respect to PNA extractibility, although the same tissues from different species behaved similarly in this respect.

Extraction of DNA. Feulgen-stained control and experimental sections of dog liver were compared visually and photometrically after 18 hours and 93 hours of extraction in 10% perchloric acid at 4°C. No differences were observed visually in the intensity of nuclear staining. Photometric measurements, expressed as extinction coefficients, of a constant circular area 6.7 μ in diameter of liver cell nuclei in these sections confirmed the visual impression (Table II). Sections of spinal cord of cat incubated at 20°C and 37°C in perchloric acid for several time intervals along with control sections in water were similarly studied. No perceptible difference in the intensity of Feulgen coloration of glial nuclei was observed between control and experimental sections incubated for 16 hours at 20°C and for 15 minutes at 37°C. After 22 hours of extraction at 20°C and 20 minutes at 37°C, however, the experimental sections showed a perceptible reduction in Feulgen staining of nuclear chromatin. Photometry of a circular area 3.3 μ in diameter of astrocyte nuclei of the grey matter confirmed the visual impressions. Although little, if any, DNA has been extracted at the time PNA is removed, it is evident that DNA is rapidly removed after this point at higher temperatures.

Extraction of protein. Sections of cat spinal cord were extracted with 10% perchloric acid for 15 minutes at 37°C, at which time PNA extraction was complete. The intensity of coloration of such sections appeared the same as that of control sections incubated in water at 37°C in Millon preparations. Photometric measurements indicated that this extraction procedure did not remove significant quantities of protein from nerve cell cytoplasm in fixed deparaffinized sections of nervous tissue, the mean extinction of the control sections being .36 ± .04 as compared with .34 ± .02 for the experimental sections.

Effect on cell structure. No alterations could be detected in sections of spinal cord and liver as a result of perchloric acid extraction at 4°C, 20°C, and 37°C.

Effect on the extinction coefficient at 2537Å. The mean extinction coefficient of cat skeletal muscle at 2537Å was unaltered by the perchloric acid extraction procedure. Photographs taken at this wave length after this procedure were identical with those taken prior to treatment. The extraction procedure likewise did not alter the E2537Å of the ventral horn cells of the spinal cord that had been pretreated with ribonuclease to remove PNA. In nerve cells, cytoplasmic PNA accounted

TABLE III. Effect of Ribonuclease and Perchloric Acid Digestion on E_{2537}Å of Skeletal Muscle Fibers and Nerve Cells (Mean ± St. Dev. N=10).

Tissue	Before treatment	After ribonuclease	After perchloric acid
Muscle	.24 ± .01		.24 ± .04
Neurones	.45 ± .02	.25 ± .03	.26 ± .02

for only 44.5% of the total E2537Å (Table III).

Discussion. The perchloric acid procedure of Ogur and Rosen suffers from several limitations as a substitute for ribonuclease in the qualitative histochemistry of nucleic acid. Its basic shortcoming is its lack of specificity for PNA. When extraction is carried out at 4°C, DNA is not removed in significant quantities even after prolonged treatment. Such treatment, however, may depolymerize DNA without extracting it, as suggested by reduced stainability with methyl green(3). Several factors affect the extractability of PNA by perchloric acid and may require either a longer period of treatment at 4°C than the 18 hours suggested by Ogur and Rosen or higher temperatures for incubation. PNA may be different chemically or physicochemically in different organs of the same animals and these differences may be reflected in differences in perchloric acid extractability. This was found to be true for nerve and liver cells of the cat and dog. Species differences in PNA also may influence extractability. Cassel(4) indeed found that 30 hours of exposure to perchloric acid was required to remove all the PNA from a species of bacteria. Fixation can affect PNA extractability as was found to be the case for nervous tissue hardened for a long period of time in formalin. Inbedding in hot paraffin also can influence PNA extractability(5). In the application of this procedure to any particular fixed tissue, therefore, it may become necessary to determine the optimum conditions for extraction with perchloric acid that will give a complete removal of PNA with little or no loss of DNA. Where one is concerned only with cytoplasmic PNA, the problem is greatly simplified, inasmuch as DNA is not normally found outside nuclei.

Perchloric acid extraction would appear to lend itself admirably to quantitative ultraviolet microabsorption spectroscopy of PNA. As has been pointed out by Caspersson(14) and others, the extinction coefficients obtained in tissue sections near the nucleic acid absorption maximum of 2600Å are only partly attributable to specific absorption by purine and pyrimidine nucleotides. Non-specific light losses incurred by reflection, refraction, and scattering of light in an optically heterogeneous medium contribute to no small extent to the extinction coefficients obtained in tissue section in the short ultraviolet region. Tissue protein, while its absorption maximum is close to 2800Å, also exhibits considerable specific absorption at 2600Å. This value may be from one-half to two-thirds or more of the E_{2800}Å for protein. Since nucleic acid in tissue sections will almost always be found in conjunction with protein, the specific absorption of the latter may contribute appreciably to the total E_{2600}Å. Pollister and Ris(10) have suggested the use of a tissue "blank" for the evaluation of the above errors and have employed hot trichloracetic acid to provide such blanks. Pollister and Leuchtenberger (15) and Hamberger and Hyden(16) used crystalline ribonuclease for this purpose. That the perchloric acid extraction procedure is well designed for this purpose is supported by the following experimental evidence. This procedure did not remove appreciable amounts of tissue protein. It did not alter cell structure in fixed sections as seen with the phase contrast microscope or in sections stained with acid dyes. Finally, it did not influence the E_{2537}Å of muscle fibers, which contain no PNA, and nerve cells in which PNA had first been removed with crystalline ribonuclease.

Summary. (1) PNA was completely extracted from fixed sections of liver by 18 hours of treatment with 10% perchloric acid at 4°C. No significant quantity of DNA was removed by such treatment even after 93 hours of incubation. (2) The above treatment is inadequate to extract PNA completely from nerve cells. However, with formalin-fixed nerve tissue, 12 hours incubation in 10% perchloric acid at 20°C and 15 minutes at 37°C was found to effect complete extraction of PNA with no loss of DNA. (3) At higher temperatures of extraction, the margin of

safety between complete removal of PNA and incipient extraction of DNA was much smaller. (4) Prolonged hardening of nervous tissue in 10% formalin rendered PNA resistant to extraction by perchloric acid. (5) Perchloric acid extraction of fixed sections of nervous tissue removed insignificant quantities of nerve cell protein. (6) Cell structure was unaltered by this extraction procedure. (7) This extraction procedure did not alter the E_{2537}Å of two tissues without PNA, skeletal muscle and ribonuclease-treated nerve cells. It was suggested that this procedure could be employed to provide tissue "blanks" in ultraviolet microabsorption spectroscopy for PNA in tissue sections.

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Determination of Polyglucose in Blood and Urine.* (19308)

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Polyglucose is a water-soluble polymer of glucose prepared by chemical polymerization. Preparations with molecular weights ranging from 3000 to 160000 have been obtained. Animal experiments have indicated that solutions are non-toxic, and may have value for use as a blood substitute in treatment of shock.†

* This research was done under the auspices of the Atomic Energy Commission.

† Material for the present studies was obtained by courtesy of the E. I. du Pont de Nemours and Co. through the Medical Research Committee of the National Research Council. This material was prepared by Dr. P. T. Mora of the Rayon Dept., Pioneering Research Laboratory, Dupont Experimental Station, Wilmington, Del. Sample P-24-14-PIa and sample P-24-52-2 were the samples used in the majority of the experiments described in this paper.

Composition of polyglucose used as basis for analytical calculations. Polyglucose, like starch and glycogen, is difficult to dry to an anhydrous condition. Air-dried preparations contain 10% or more of moisture. After drying 8 days at 80° at atmospheric pressure, a preparation reached constant weight. Its carbon content then was 42.37%, corresponding to $(C_6H_{10}O_5)_2 \cdot H_2O$ (theory, 42.1% carbon), rather than to $C_6H_{10}O_5$ (44.44% carbon). It is uncertain whether the half molecule of water per $C_6H_{10}O_5$ unit in the dried preparation was moisture that could not be driven off under the conditions used, or whether it was a part of the molecular structure. In starch and glycogen Dumazert(3) has found the same composition (C = 42.2 to 42.4%) when these carbohydrates were dried at 80° under less than 1 mm pressure. His