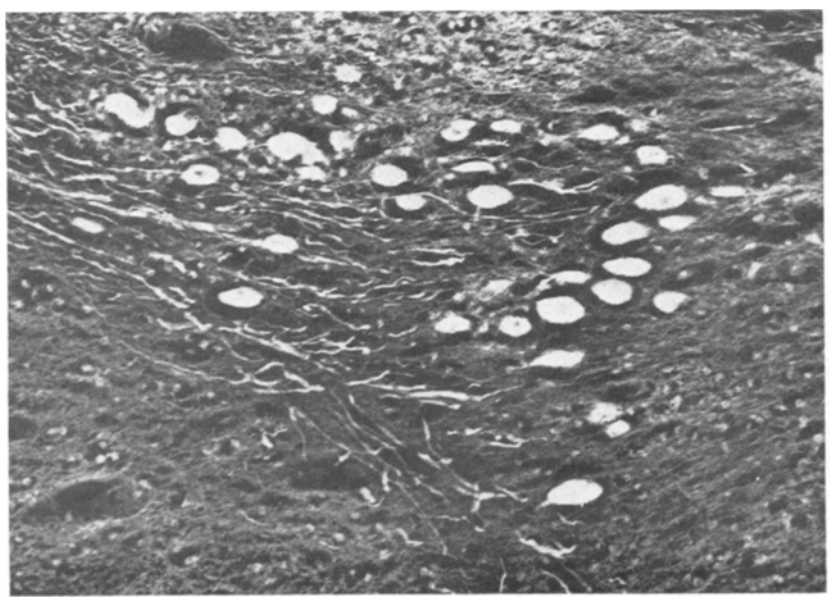
The Technic of X-Ray Histochemistry Applied to Neurohistology[1]

In order to obtain a more complete X-ray picture of the microcirculatory pattern in the brain than can be obtained by injection technics, a series of experiments was undertaken to try to stain the smaller vessels for contact and projection X-ray microscopy[2,3]. For this purpose some of the standard histochemical reactions developed for optical microscopy were used, and where necessary modified, to produce X-ray images of the vessels or neurones alone, or in combination, to show their interrelation.

In the course of these experiments on different brain regions of both rats and humans, it was noticed that major cellular and vascular patterns, groups, and distributions frequently stood out with more striking contrast in an X-ray micrograph than in a conventional photomicrograph. In Figure 1, a parasagittal section of rat brain stained for neurones, the general distribution of neurones throughout the brain stem is quite clear; major fibre bundles, mostly unstained and therefore radiolucent, appear dark. The granule cell layer and the roof nuclei of the cerebellum (upper right), 2 portions of the large-celled mesencephalic nucleus of V (upper left, and left of centre just below fibres of the superior cerebellar peduncle), and the fibre bundle of the genu of the facial nerve (right of centre) can all be identified, and along the bottom of the picture from left to right appear the large nuclear mass of the pons, the fibres of the cerebrospinal tract, the nucleus of the trapezoid body and the inferior olivary nucleus. Two higher power pictures of the 2 portions of the mesencephalic nucleus show the

a

Fig. 1. Rat hindbrain, paraffin section, approximately 20 μ. Adaptation of Gomori acid phosphatase technic at pH 5. (a) Contact microradiograph, Philips CMR-5 unit. Ilford high resolution emulsion. × 19.



b

Fig. 1. (b) Contact microradiograph, Philips CMR-5 unit. Spectroscopic emulsion (Kodak 649-0). × 200.

[1] This work formed part of the research submitted for a Master of Science degree at Dalhousie University. It was supported in part by MRC Grant No. MT-752 and carried out under the supervision and with the kind assistance of Prof. R. L. DE C. H. SAUNDERS. Thanks are due to Mr. V. CARVALHO and Mrs. PAT TROYER for X-ray and photographic assistance.

[2] R. L. DE C. H. SAUNDERS, Can. J. Surg. *5*, 299 (1962).

[3] R. L. DE C. H. SAUNDERS and R. V. ELY, in *X-Ray Optics and Microanalysis* (Hermann, Paris 1965), p. 642.

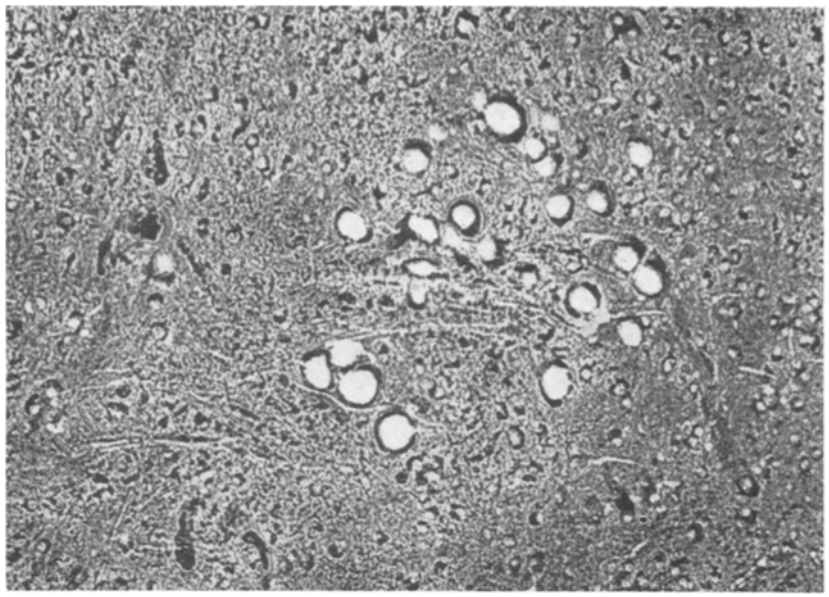


Fig. 1. (c) Projection microradiograph, Cosslett-Nixon XM-30 unit. Ilford high resolution emulsion. × 230.

Fig. 1. Note that the wide spectrum radiation from the tungsten target of the contact unit at 3 kV (b) is more penetrating than the monochromatic aluminium radiation at 15 kV used in the projection microscope (c); (c) therefore shows less contrast than (b), but more of the delicate tissue detail around the neurones.

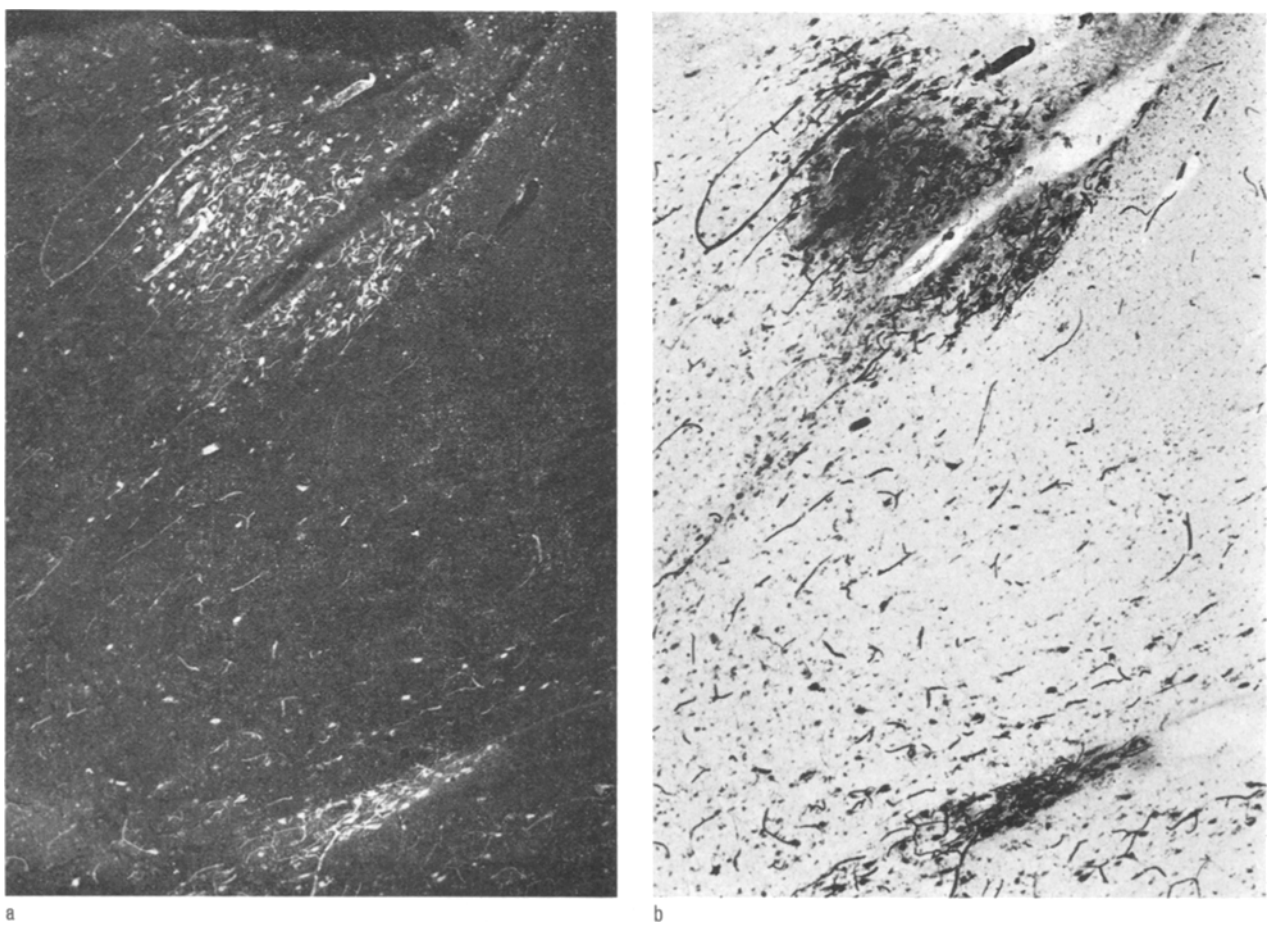


Fig. 2. Human hypothalamus, supra-optic area, frozen section, approximately 35 µ. Direct lead incubation at pH 7, after Gomori. (a) Contact microradiograph, Philips CMR-5 unit, high resolution emulsion. × 50. (b) Microphotograph, same area (Zeiss photomicroscope).

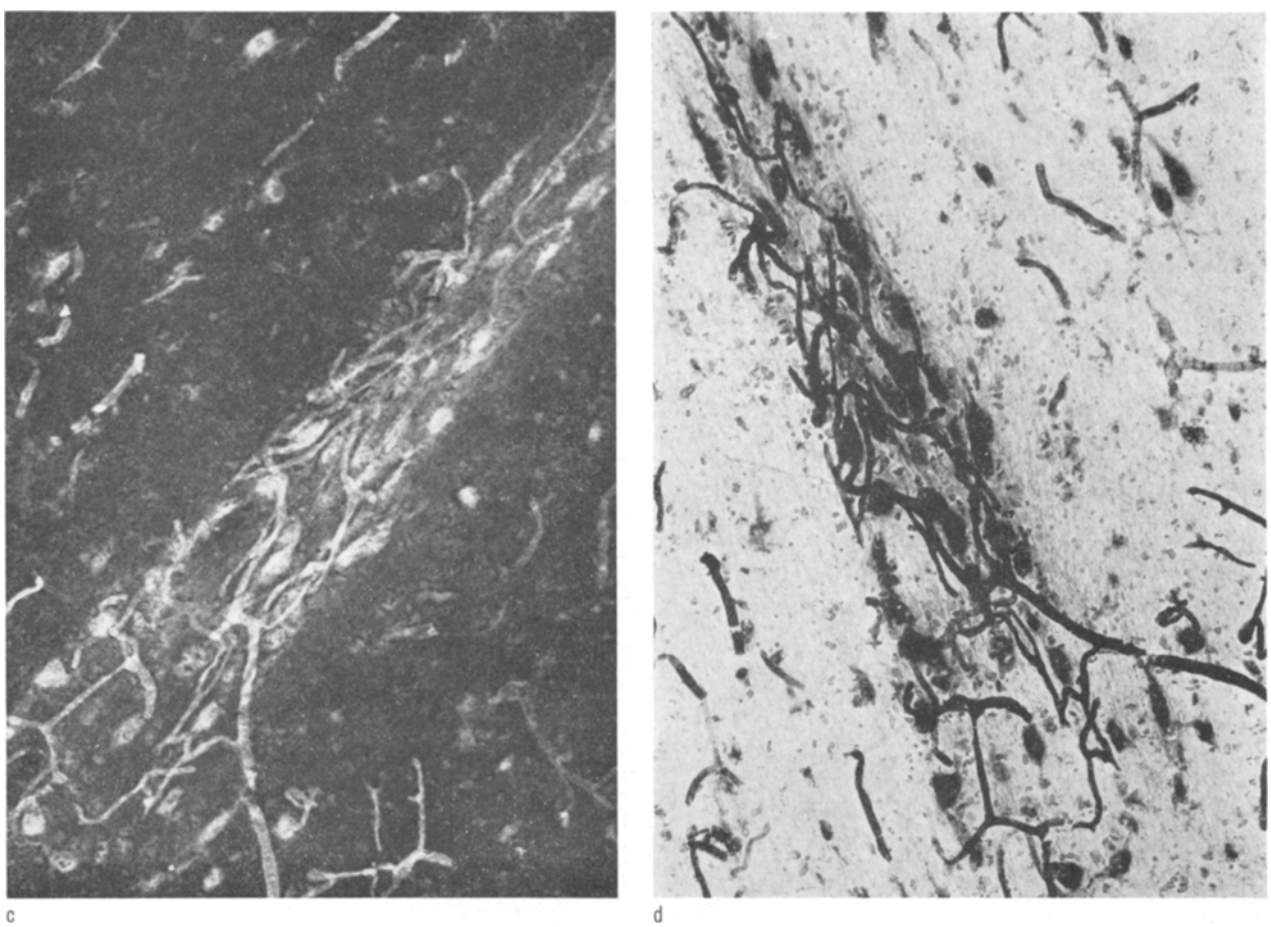
degree of detail that can be obtained with different X-ray and photographic technics. Figure 2a is an X-ray micrograph of a human hypothalamus in parasagittal section in which the supra-optic nucleus appears on either side of the dark, unstained fibre bundle of the optic tract. In this preparation both the blood vessels and the nerve cells have been stained. Figure 2c shows the detail at high power; 2b and d are microphotographs of the same specimen for comparison. Figure 3a shows some neurones and their (lightly stained) related capillaries in the central part of human hypothalamus; 3b is a microphotograph of the same features; 3c gives a low power X-ray survey of the area.

X-rays are absorbed by heavy metals and many histochemical technics originally developed for light microscopy result in metal deposits, or may be altered to do so. In the preparations shown, nerve cells and blood vessels were both stained by adaptations of standard histochemical methods that demonstrate the sites of activity of the non-specific phosphatase enzymes. In the case of the nerve cells, the perikaryon, or cell body, contains appreciable amounts of the acid phosphatase enzyme, for which Gomori developed the classic series of metallic reactions resulting in a final product of insoluble lead sulfide at the enzyme's site[4]. By trial and error the incubation time may be adjusted so that sufficient lead sulfide is precipitated to give well-defined X-ray absorption without allowing diffusion to blur the image of the cell. In the case of the blood vessels, the endothelial cells of their walls contain alkaline phosphatase, and a number of methods using this enzyme's activity and based on Gomori's metal technics have been used to outline the vessels with lead[5]. Figures 2 and 3 show the picture resulting from 2 of these methods: Figure 2 is a direct lead incubation at pH 7 designed to show both cells and vessels by compromising between the two optimal pH's of the acid and alkaline phosphatases (pH 5 and pH 9), and Figure 3, a sequence of 2 direct lead incubations, one at pH 5 for cells, followed by one at pH 9 for vessels. These figures emphasize the fact that in preparations of this type the same piece of tissue can be examined by both, light and X-ray microscopy.

The above examples may be mainly useful as morphological demonstrations; similar preparations might fit well into the radiographic and microangiographic studies of areas and tissues when it is desired to extend the range of magnification and detail beyond the normal limit of standard radiography. The readily available commercial

[4] G. GOMORI, *Microscopic Histochemistry* (University Chicago Press, Chicago 1952).

[5] MARY A. BELL, Thesis, Dalhousie University 1969.



c d

Fig. 2. (c) Contact microradiograph, Philips CMR-5 unit, spectroscopic emulsion. × 200. (d) Microphotograph, same area.

contact microradiograph units and the specially constructed X-ray projection microscopes being used in some research laboratories[2,3] now make it possible to obtain X-ray images through approximately the same range of magnification that is currently used by the light microscope. Two properties of X-rays that light does not share make such X-ray images worth considering: (1) X-rays can penetrate thicker and more opaque specimens than light can, and (2) X-rays, especially when of the characteristic monochromatic type, are absorbed in a highly

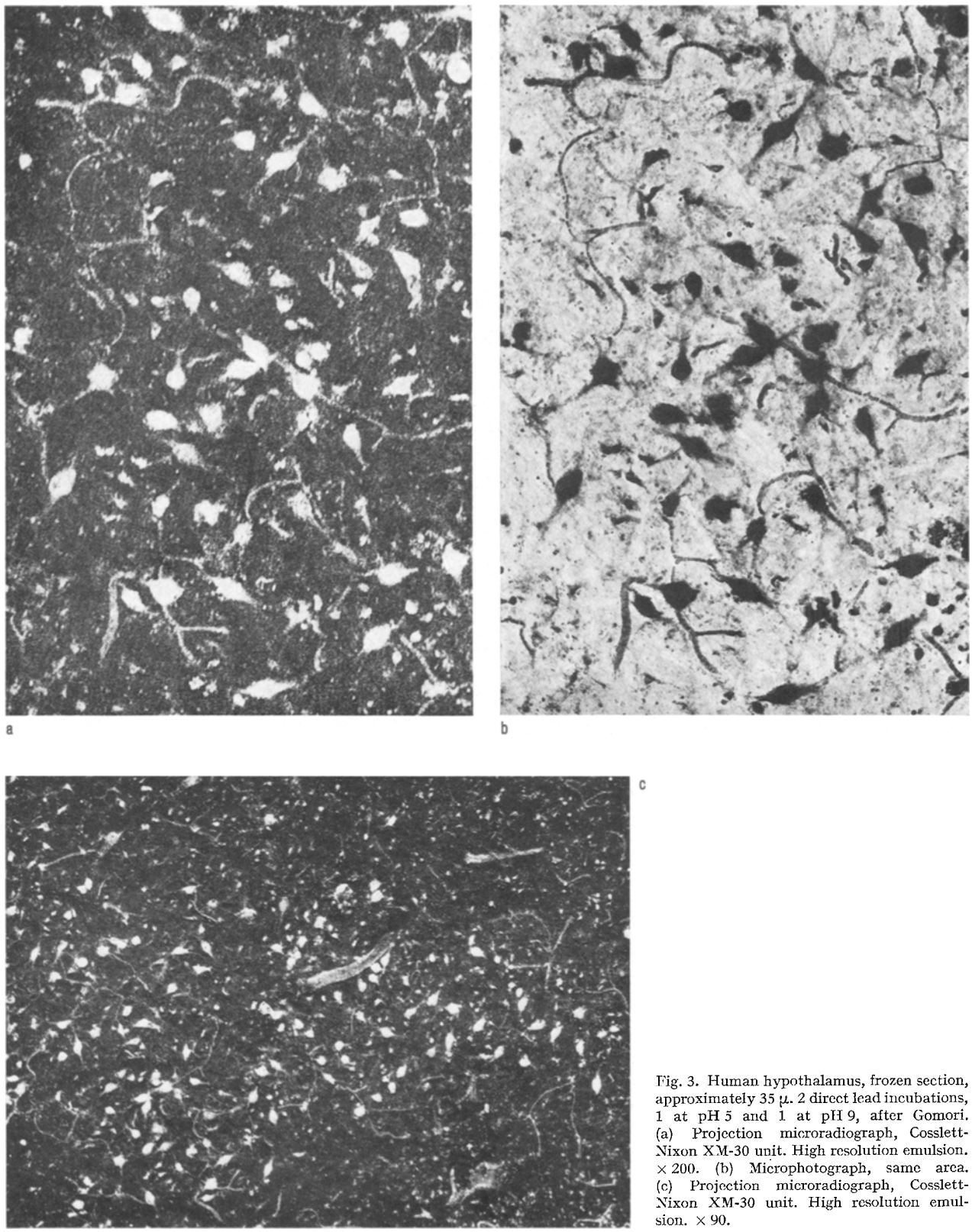


Fig. 3. Human hypothalamus, frozen section, approximately 35 μ. 2 direct lead incubations, 1 at pH 5 and 1 at pH 9, after Gomori. (a) Projection microradiograph, Cosslett-Nixon XM-30 unit. High resolution emulsion. × 200. (b) Microphotograph, same area. (c) Projection microradiograph, Cosslett-Nixon XM-30 unit. High resolution emulsion. × 90.

specific way that can yield much qualitative and quantitative information about the absorbing element. This last property suggests that the field of X-ray histochemistry, in addition to providing a new means for morphological demonstrations of structure and biochemical distributions, may make important contributions to qualitative and quantitative chemical analyses at both, tissue and cellular levels. Biochemistry must often sacrifice structure to obtain quantitative chemical measurements; histochemistry often preserves morphology at the expense of quantitative accuracy; perhaps X-rays can help to bridge this gap.

Zusammenfassung. Demonstrationsvergleich röntgenmikroskopisch behandelter Zellen und Blutgefässe von Ratten sowie von menschlichem Gehirngewebe mit optischen Standardmethoden, wobei Gomoris Bleisulfidtechnik zur Bestimmung der Phosphataseenzymaktivität angewandt wurde. Die Brauchbarkeit der histochemischen Technik für die Röntgenologie wird diskutiert.

MARY A. BELL

Department of Anatomy, Dalhousie University, Halifax (Nova Scotia, Canada), 28 February 1969.

Steroide und Fibroblastenwachstum[1]

Widersprüchliche Ergebnisse über den Einfluss verschiedener Kortikosteroide auf das Wachstum von Fibroblasten waren Veranlassung zur Durchführung der vorliegenden Untersuchungsreihe. Während ein Teil der Untersucher über die Wachstumshemmung von Fibroblasten unter Kortisoneinwirkung berichten, fanden andere[2–4] eine Wachstumsförderung.

Es wurde der permanente Mäusefibroblastenstamm «L» auf Hühnerembryonalextrakt, Kälberserum und Hankscher Salzlösung in Vierkantflaschen gezüchtet (Wechsel des Mediums jeden 2. Tag, Teilung der Kulturen jeden 4. Tag). Durch Vorversuche wurden zwei stark wirksame Steroide (Prednisolon[5] und Betametason[6]) ermittelt. 24 h nach Teilung der Kulturen Zusatz von zwei Verdünnungsreihen: Prednisolon 0,1–1–10–100–1000 γ/ml und Betametason 0,01–0,1–1–10–100 γ/ml 18 Stunden nach Einwirkung der Steroide Zugabe von 1 μc/ml ^{3}H-Thymidin und weitere 6 Stunden später Arretierung des Zellwachstums: Ablösung der Zellen mit 0,06% Trypsin, Waschung mit Hankscher Salzlösung, Fixierung mit Eisessig-Alkohol 1:3, Färbung mit Orcein. Die Autoradiographie erfolgte mit Kodak AR 10 bei einer Expositionsdauer von 10 Tagen. Von jeder Kultur wurden 10000 Zellen differenziert.

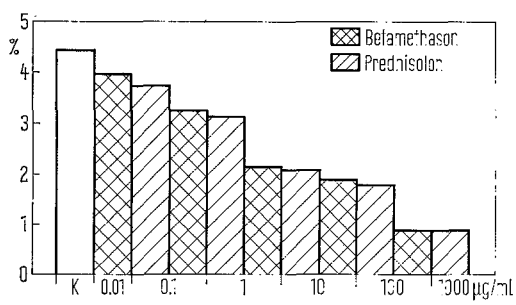
Abbildung 1 gibt die Ergebnisse wieder: Es besteht eine lineare Beziehung zwischen der Steroidkonzentration im Medium und dem Zellwachstum. Auch bei den niedrigsten Dosierungen ist eine deutliche Hemmwirkung nachzuweisen. Entsprechend der Steroidkonzentration fanden sich bei den behandelten Kulturen weniger markierte Zellkerne als bei den unbehandelten Kontrollen. Dieser Befund steht im Gegensatz zu den Ergebnissen anderer Autoren. FARNES et al.[2] fanden einen Anstieg der Zellproliferation von fibroblastenähnlichen Makrophagen in menschlichen Knochenmarkkulturen bei Prednisolonkonzentrationen zwischen 0,5 und 50 γ/ml Medium. MACIEIRA[3], RASCHE et al.[4] sahen bei hohen Steroidkonzentrationen eine Wachstumshemmung, bei niedrigen Dosen jedoch eine wachstumsanregende Wirkung. Andere Autoren[7] führten diesen Befund auf eine Arretierung der Mitosen zurück, da bei niedrigen Steroiddosen der übrige Zellzyklus noch wenig gestört ist. LETTRE et al.[8] konnten dagegen keine Wirkung der Steroide auf die Mitose nachweisen.

Als zweiten Befund ergab die vorliegende Untersuchung eine zehnmal stärkere Wirkung des Betametasons im Vergleich zu Prednisolon, während andere Autoren differente Angaben machen[9]. Weitere Untersuchungen über die Wirkung von Steroiden auf andere Gewebe in vitro sind im Gange.

Summary. To clarify the contradictory results concerning the influence of different corticosteroids on growth of fibroblasts, the rate proliferation of L-cells under increasing doses of prednisolone and β-methazone was studied by measuring ^{3}H-thymidine uptake. Growth was inhibited in a linear fashion by an increasing concentration of steroids. β-methazone had a 10 times stronger effect compared with an equal quantity of prednisolone.

O. WIESER und A. H. TAIFOUR

Deutsches Krebsforschungszentrum, Institut für experimentelle Pathologie, D-69 Heidelberg 1 (Deutschland), 8. April 1969.



Prozentueller Anteil markierter Fibroblasten.

[1] Mit Unterstützung der Strebel-Stiftung Mannheim.
[2] P. FARNES und B. E. BARKER, Metabolism *14*, 75 (1965).
[3] C. A. MACIEIRA, Experientia *22*, 390 (1961).
[4] B. RASCHE und W. T. ULMER, Z. Zellforsch. *84*, 506 (1968).
[5] Solu-Decortin-H®.
[6] Celestan soluble®.
[7] TH. F. DOUGHERTY, D. L. BERLINER und M. L. BERLINER, Metabolism *10*, 966 (1961).
[8] H. LETTRE, C. LANDSCHÜTZ und J. NOBEL, Klin. Wschr. *5*, 555 (1951).
[9] H. KAISER, *Kortisonderivate in Klinik und Praxis* (Georg Thieme, Stuttgart 1965).