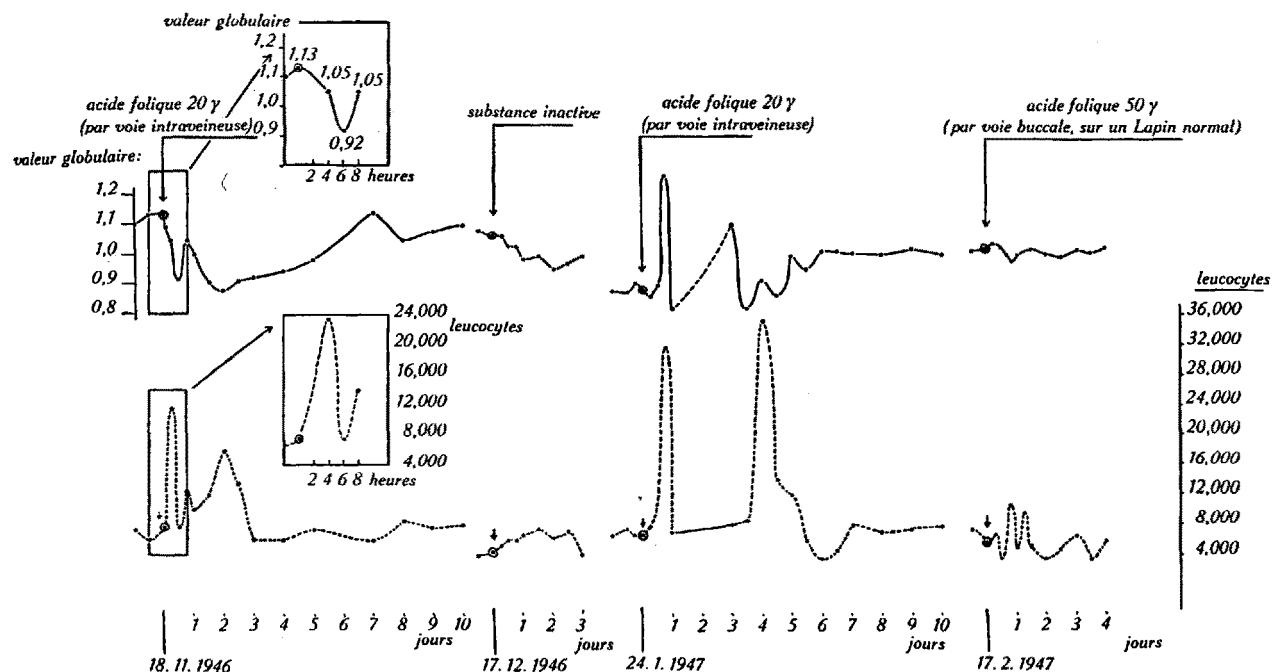


Le 18 novembre 1946, les 24 janvier, 17 février et 4 mars 1947, cette lapine a reçu 20 γ d'une solution bicarbonatée d'acide folique¹ dans les veines. Chaque fois, cette injection a provoqué un déséquilibre sanguin dont l'ampleur dépasse de beaucoup la marge des variations spontanées. Ce déséquilibre intéresse au moins deux des éléments du sang: les érythrocytes et les leucocytes. Pour ces derniers, l'action est particulièrement marquée; elle n'est en aucune manière sous la dépendance des phénomènes digestifs: notre lapine était restée à jeun tout le jour de l'injection. Le leucocytose atteint chaque fois des valeurs élevées; qui croissent à

l'injection, apparaissant irrégulièrement entre la 2^{me} et la 8^{me} heure, il y a un premier phénomène souvent discret, toujours très fugace; la leucocytose en particulier ne dure pas plus de une à deux heures et échappe facilement à l'observation. Ceci est illustré par les deux petites courbes encadrées, à l'échelle horaire. Ce phénomène est suivi d'un deuxième qui est souvent plus intense ou tout au moins plus prolongé. Il apparaît dans le courant de la 2^{me}, de la 3^{me} ou même de la 4^{me} journée. Sur cette réaction viennent quelque fois s'en greffer d'autres: par leur intensité, elles ne semblent pas être un reliquat de la réaction initiale, mais cons-



chaque injection. Les maxima observés ont été successivement de 23000, 35000, 70000 et 84000 éléments par mm³. En ce qui concerne les érythrocytes, c'est la charge en hémoglobine, la valeur globulaire qui varie. Lorsqu'on porte en courbe ces variations de la valeur globulaire on obtient des figures qui diffèrent d'un animal à l'autre et chez le même animal au cours d'essais successifs: ou bien la valeur globulaire augmente ou bien elle diminue; elle peut aussi baisser après avoir augmenté. Ce qui compte, c'est la valeur et la rapidité de la dénivellation. Avant de retourner à la valeur initiale, ce qui se fait au bout de 8 jours, environ, on observe une suite d'oscillations d'amplitude décroissante.

Ces effets semblent absolument généraux. Nous les avons observés sur 8 lapins d'âge et de sexe différents que nous avons traités avec des doses d'acide folique variant de la dose seuil (10 γ) à 130 γ . Les fortes doses, pas plus que les doses répétées, ne semblent augmenter ni la durée ni l'intensité des réponses. Donné par voie buccale, l'acide folique semble perdre toute son action sur l'hématopoïèse normale: avec 50 γ nous n'avons eu aucune variation de la valeur globulaire et les variations des leucocytes étaient très peu significatives (voir l'essai à droite de la figure).

A ne considérer que le sang circulant, l'acide folique paraît agir d'une manière complexe. Dans la journée de

titrer un phénomène indépendant. Il est vraisemblable que la persistance de ce déséquilibre sanguin n'est pas due à l'acide folique lui même mais à des corps résultant de sa transformation dans l'organisme.

Nous nous préoccupons de savoir si ces phénomènes sont liés à des modifications des organes hématopoïétiques ou à une simple mobilisation des éléments sanguins déjà libérés.

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Summary

After intravenous injections of 10 γ or more of folic acid to normal rabbits the following phenomena are observed in the blood: a rapid variation of the colorimetric index; a marked leucocytosis. The figure illustrates these phenomena which are very fugitive, particularly the leucocytosis.

Histochemical analysis by X-rays of long wavelengths

In a series of works LAMARQUE¹ and co-workers and others showed that it is possible to obtain, in relatively considerable enlargement, clear and distinct microradio-

¹ Nous tenons à remercier encore une fois Monsieur le Professeur C. A. ELVEHJEM pour l'envoi d'un échantillon d'acide folique synthétique.

¹ P. LAMARQUE, J. Radiol. et Electr. 20, 325 (1936); J. Belge Radiol. 27, 109 (1938).

grams of thin biological tissue sections. In 1946 ENGSTRÖM^{1,2} showed that, by means of X-ray absorption spectrography combined with microradiography, it is possible to make a quantitative elementary analysis of certain elements within very small parts of a biological tissue, in certain cases of the individual cell. This analytical procedure is based upon the measurement of the absorption of monochromatic X-rays within a very small surface of an object, e.g. a microtome section of a tissue. By repeating the measurement procedure in a series of wavelengths lying on different sides of a long wave absorption edge for the sought-for element, the amount of the latter can be calculated. For the theoretical basis of the analysis reference should be made to ENGSTRÖM².

Owing to the fact that the biologically interesting elements have low atomic numbers, their characteristic absorption edges lie within the extremely soft X-ray region, which involves technical difficulties, as the apparatus employed for obtaining long wave monochromatic X-rays is rather complicated.

For measurements where very great accuracy is not necessary, and for microradiography with long wave filtered primary radiation, the simple apparatus described below is appropriate. Fig. 1A shows a longitudinal section through the apparatus. The anode *A*, the position of which is adjustable in high vacuum, is isolated from the rest of the X-ray tube by means of a porcelain tube and has positive voltage. The focus is coated with platinum. The filament, which is oxide-coated, is arranged to give a line focus. The anode *A*, the tube body *B*, and the cathode *C*, are water-cooled. All the vacuum packings are effected with rubber. Fig. 1B shows a cross section through the tube as used for microradiography with primary radiation. The tube is evacuated to high vacuum through the channel *V* (see Fig. 1C) and is closed in the front by the aluminium foil *F*, which serves at the same time as a filter. The primary radiation thus passes the foil and impinges subsequently on the specimen and the film, which are in good contact with each other at *S*. The specimen chamber is evacuated by means of a special vacuum system through *E*. With a reasonably long exposure time, it is possible to obtain good microradiograms in wave-lengths up to c. 30 Å (c. 400 V) on a fine-grained film e.g. Ilford High Resolution or Eastman Type 548-0.

With the use of characteristic secondary K-radiation the tube is employed according to Fig. 1C. Opposite the emergence aperture for the primary radiation is placed a plug *H*, on which is soldered the metal or rubbed in the element whose characteristic radiation is desired. The specimen and the film are placed at *P*, and the evacuation takes place through *E*.

The characteristic radiation is relatively monochromatic when K-radiation is employed, and L—and longer wave radiation is filtered off. In the following table is shown a series of analysis results, when secondary radiation was employed. In micro-cuvettes holding 1 mm³ which are inserted at the place of the specimen *P* in Fig. 1C, the amount of iron in water solutions of known iron-content was determined. The analysis was made at normal pressure.

Secondary radiation from 28 Ni and 27 Co was employed. These K-radiations have wave-lengths lying on either side of the K-absorption edge and iron, 1.74 Å. The mass absorption coefficients for secondary radiation from Ni and Co in iron are 314 and 67 respectively. The

absorption of the X-rays in the specimen was measured photographically-photometrically, and the calculations made according to ENGSTRÖM¹.

Fe taken 10 ⁻⁶ g/mm ²	Fe found 10 ⁻⁶ g/mm ²	Error %
13.0	14.4	+ 10.8
23.6	23.8	+ 0.8
23.6	21.6	- 8.5
23.3	23.1	- 0.9
46.3	45.7	- 1.3

A histological application is as follows: In Fig. 2 is shown a microradiogram of a section of skin, taken with filtered primary radiation, with a tube voltage of 800 V, corresponding to an effective wave-length of ≈ 20 Å. The magnification is 70 times, and it appears from the photograph that, when this extremely soft radiation is employed, in respect to X-ray absorption the skin

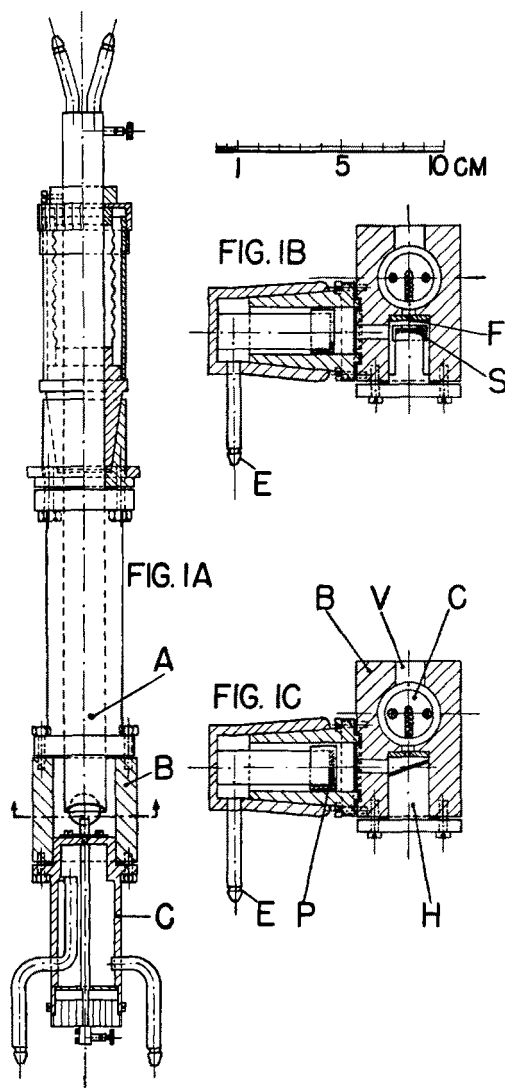


Fig. 1. X-ray tube for primary and secondary microradiography.

¹ A. ENGSTRÖM, *Nature* 158, 664 (1946).

² A. ENGSTRÖM, *Acta Radiol.* (Stockholm), Suppl. LXIII (1946).

¹ A. ENGSTRÖM, *Acta Radiol.* (Stockholm), Suppl. LXIII (1946).

consists of layers sharply delimited from each other, one outer, more strongly absorbing one, corresponding to the Stratum Corneum, and one inner, more weakly absorbing one, corresponding to the other layers of the skin. The border between the skin and the underlying connective tissue appears clearly. If, instead, an exposure is made of this section with characteristic secondary radiation from 16 S and 17 Cl with K-radiation

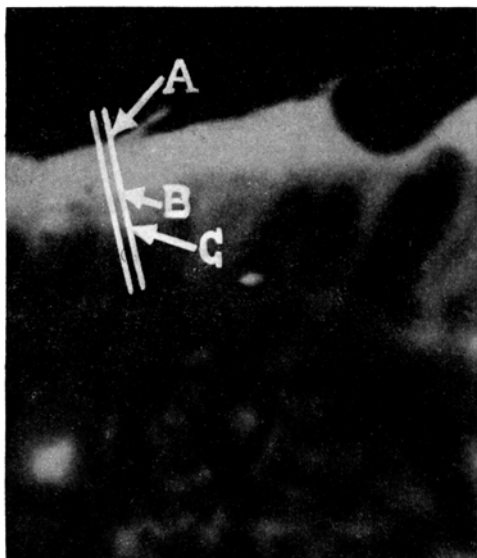


Fig. 2. Microradiogram of a section of skin.

5.36 and 4.72 Å respectively, the sulphur content in different parts of the skin can be determined. The K-absorption edge for sulphur has the wave length 5.00 Å. The results of such a sulphur determination appear from Fig. 3. The absorption of the X-rays was measured by the photographic-photometric method. For measurements of the photographic density which always takes place directly on the X-ray film, the technique and apparatus, worked out by CASPERSSON¹ for measuring the light absorption in individual cell-details, was employed. The size of the measurement surface was 1000 μ^2 , owing to the fact that even exposures at 5.36 and 4.72 Å respectively revealed a division in homogeneous layers of the skin. The amount of sulphur is given in 10^{-10}

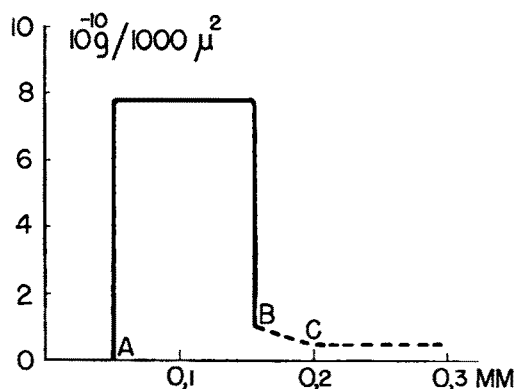


Fig. 3. Determination of sulphur in different layers in skin. Compare Fig. 2.

g/1000 μ^2 , and the measurement surface is shifted along the line A-B-C in Fig. 2. The thickness of the section is 15 μ .

With the filtered primary radiation, measurements were also made in the region of the K-absorption edges of oxygen and nitrogen, but these measurements could not give quantitative data direct, as the breadth of the radiation band was too great. In the case of exposures with these extremely soft X-rays, very small amounts of high atomic elements also stand out in contrast to, e.g., ordinary tissue. By allowing certain substances in the tissue to react with heavy elements, e.g. glycogen with iodine, nucleic acids with lanthanum ions, protein with phosphotungstic acid, etc., and then determining these heavy elements in the cell or a part of the tissue section with long wave primary radiation, a kind of "X-ray staining" is obtained. Thus it seems possible, at least semi-quantitatively, to determine in this manner certain groups of substances on a histo- and cytochemical scale.

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A. ENGSTRÖM and B. LINDSTRÖM

Department for Cell Research, Karolinska Institutet, Stockholm, April 6, 1947.

Résumé

Description d'un appareil pour l'enregistrement de microradiogrammes avec des rayons X extrêmement mous. Il convient aussi bien à l'emploi du rayonnement primaire filtré qu'à celui du rayonnement secondaire caractéristique. Ce dernier rend possible une analyse cyto- et histochimique au moins semi-quantitative.

Les effets de l'autotransplantation préportale de la thyroïde chez la Rate

Les recherches récentes de GOLDEN et SEVERINGHAUS¹, de ENGEL², de LIPSCHÜTZ³, ont montré que les transplants d'ovaire ont, chez la Rate, la Lapine ou la Cobaye, une action très différente sur l'équilibre endocrinien, suivant qu'ils sont transplantés soit dans la région axillaire ou les muscles du dos, soit dans le mésentère ou dans la rate. Dans ce dernier cas l'animal prend le type ovariectomisé, car les produits sécrétés par le transplant ovarien sont drainés vers le parenchyme hépatique qui a la propriété de les inactiver, tant *in vitro* qu'*in vivo* (SILBERSTEIN⁴, RONDONI⁵, ZONDEK⁶, ISRAEL⁷, BISKIND⁸, HELLER⁹, SCHILLER¹⁰).

Or, comme les hormones œstrogènes naturelles, la thyroxine est plus active par voie parentérale que *per os*. Il nous a paru intéressant de suivre le sort de thyroïdes transplantées en amont du tronc porte. MANLEY et

¹ GOLDEN, SEVERINGHAUS, Inactivation of estrogenic hormone of the ovary by the liver, *Proc. Soc. exp. Biol. Med.* 39, 361 (1938).

² ENGEL, A study of inactivation of ovarian hormone by the liver, *Endocrinology* 35, 70 (1944).

³ LIPSCHÜTZ, Study of the gonadotrophic activity of the hypophysis *in situ*, *Nature* 157, 551 (1946).

⁴ SILBERSTEIN, MOLNÁR, ENGEL, *Klin. Wschr.* 12, 1694 (1933).

⁵ RONDONI, CARMINATI, CORBELLINI, *Z. Physiol.* 24, 171 (1934).

⁶ ZONDEK, *Skand. Arch. Physiol.* 70, 133 (1934).

⁷ ISRAEL, MERANZI, JOHNSTON, *Am. J. med. Sci.* 194, 835 (1937).

⁸ BISKIND, *Endocrinology* 28, 894 (1941).

⁹ HELLER, *Science* 99, 145 (1944).

¹⁰ SCHILLER, *Endocrinology* 36, 715 (1945).

¹ T. CASPERSSON, *J. Roy. Micr. Soc.* 60, 8 (1940).