Table I

Shoot and root length of plants treated with different concentration of 6-amino undecane-HCl. Experiment with flowing nutrient solution; age of the plants at the start three days. Average of 12 plants, lengths in mm of first leaf and three initial roots.

Concentration	2 days old		4 days old		6 days old	
	shoots	roots	shoots	roots	shoots	roots
0	44·4 ± 1·2	53·8 ± 1·5	96·5 ± 2·3	92·1 ± 1·7	149·1 ± 2·0	121·1 ± 2·5
10^{-6}	42·4 ± 1·5	49·8 ± 1·5	93·9 ± 1·8	88·7 ± 1·5	142·7 ± 2·2	116·9 ± 2·3
10^{-5}	39·0 ± 1·2	46·7 ± 1·4	85·7 ± 2·4	77·4 ± 2·1	127·0 ± 2·6	104·4 ± 2.7
10^{-4}	35·9 ± 1·2	48·8 ± 1·4	67·4 ± 1·7	74·3 ± 1·5	94·8 ± 2·9	95·2 ± 2·1

In 10^{-4} mol around the fifth day the leaf bases started wilting locally and the leaves bent to one side. At the same stage the roots showed some brownish spots on their surfaces. A microscopic investigation of the latter effect showed that the amide particularly affected the trichoblast cells of the elongation zone, causing a coagulation of the cytoplasm. In the leaves the situation was different, there the amide produced a reversible loss of water, causing folding of the walls of the chlorenchyma and a plasmolysis of the parenchyma of the bundle sheaths.

By the application of staining and plasmolytic methods the vitality of the treated shoot and root cells was studied. Plasmolysis and staining times were considerably shorter than those of the controls. This fact revealed an increased permeability of the cells.

Transpiration measurements did not show any significant difference between treated and untreated plants (Table II) although there was a decrease in the water content of the leaves. From an analysis of the water balance it can be concluded that this decrease depended upon a reduced water supply through the roots. This results in a wilting, localized to the most rapidly elongating part of the leaf.

Table II

The transpiration of plants treated with 6-amino undecane-HCl. Values given in mg/20 min. and means of 5 expects.

Age of the plants	Transpiration mg		
	Control	$3 \cdot 10^{-5}$ mol/l	10^{-4} mol/l
4 days	6·9	6·1	6·1
5 days	6·3	6·1	5·4

From the above mentioned observations the following conclusions can be drawn: the loss of water in the zone of cell elongation together with the increased permeability indicate some specific cytoplasmic action of the amide which is different from that of the homologous acids.

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Botanical Laboratory, University of Lund, and Department of General Botany, University of Istanbul, October 25, 1950.

Zusammenfassung

Eine Untersuchung der physiologischen Wirkungen von 6-Amino-undekan-HCl führte zu folgenden Ergebnissen:

1. Wurzel- und Sproßwachstum von Weizenkeimlingen wurde in Lösungen verschiedener Konzentration während 6 Beobachtungstagen in steigendem Maße gehemmt. In 10^{-4} mol. trat in den Trichoblasten der Wurzelepidermis Plasmakontraktion auf. Gleichzeitig begannen infolge der ungenügenden Wasserversorgung die Blattblasen zu welken, und die Blätter sanken seitlich herab.

2. Auf Grund von Plasmolyse- und Färbungsversuchen konnte auf eine Erhöhung der Plasmapermeabilität geschlossen werden.

3. Eine eindeutige Beeinflussung der Transpirationsintensität war nicht nachzuweisen.

Human Isolated Chromosomes. Normal and Pathological Chromosomes of Leucocytes

I have been studying the chromosomes of the blood cells[1] for the last few years with recent staining techniques. With reference to the white series, my investigations have been directed towards the leukaemic cell[2]. Proofs have been obtained in several mitoses of leukaemic cells of a characteristic appearance of the chromosomes, which are found to be very thick with a homogeneous length, strongly stained and paired two by two, recalling a meiotic stage. Besides these mitoses, other were seen with numerical alterations of the chromosomic set. Further investigations with histo-chemical methods revealed a typical appearance of the leukaemic chromosomes, since they stained more strongly with the Feulgen reaction than those of the normal white cells[3]. This is in agreement with what has been shown afterwards by authors[4] using quantitative methods. Since the methods I followed did not allow to note differences in the nuclei of the cells during the resting stage, investigations were directed towards the recent methods of isolation of the chromosomes. While studies have already appeared concerning isolated chromosomes of mammals, none at all is known for human chromosomes, since the requirement for all methods of isolation is to work with a fair quantity of fresh tissue[5].

The only way of obtaining a certain quantity of fresh human cells was connected with the chance of separating quickly the white elements from the red ones.

Among the various means proposed to quicken the speed of sedimentation (starch, gum arabic, gelatine, fibrinogen) I chose gum arabic for the two following reasons:

[1] E. POLLI, Arch. Sci. Med. *82*, 425 (1946); Atti 7° Cong. Soc. It. Ematol. 333 (1947); Atti Soc. Lomb. Sci. Med. Biol. *1*, 7 (1946).

[2] E. POLLI, Ann. Biol. norm. e pat. *1*, 4 (1947); Boll. Soc. It. Biol. Sper. *25*, 48 (1949).

[3] E. POLLI, Atti Cong. Soc. It. Med. Int. *48*, 253 (1947); Exp. Cell Res. *1*, 460 (1950).

[4] B. THORELL, Acta Med. Scand. *129*, Suppl. 200 (1947). – M. L. PETERMANN and E. J. MASON, Proc. Soc. Exp. Biol. a. Med. *69*, 542 (1948).

[5] A. CLAUDE, Harvey Lectures, Series *43* (1947/48). – A. E. MIRSKY and H. RIS, J. Gen. Physiol. *31*, 1 (1947).

(1) The speed of sedimentation is not a linear, but an exponential function of the concentration of gum arabic[1].

(2) Whereas, with gelatine the agglomerating power on the red corpuscles increases when the temperature is raised, the opposite takes place with gum arabic[2]. This is of the utmost importance for those investigations that have to be carried out at a low temperature. Following this course there was no chance of damaging the nuclei of the white cells, this being proved by many control experiments with leukaemic subjects. In fact on these there is a high speed of sedimentation of the erythrocytes, and the controls carried out with or without gum showed no chances of variation in the appearance of the white cells examined.

Sedimentation of the cells was obtained by mixing (in the syringe used for taking the sample) one part of the 2% solution of gum arabic containing also 3·8% of sodium citrate, with four parts of venous blood. The blood thus citrated was put in test tubes slanted on ground ice. Sedimentation took place in an ice chest at 0–1°.

For the investigations, I used samples of 3–5 liters of normal human blood and 300–500 cm³ of leukaemic blood.

After red cell sedimentation, the supernate was centrifuged at 0–1°. The sediment contained large quantities of white corpuscles, but also platelets and red cells. In the first experiments haemolysis was carried out with saponine, which fixes itself electively on the walls of the red corpuscles (five drops of Merk's 0·04% in 10 cm³). Afterwards it was found that haemolysis was not necessary and it was therefore neglected. After many washings with NaCl 0·14 M, according to MIRSKY'S technique, using a small molybdenum steel Waring blendor with a cooling chamber, I proceeded to isolate the chromosomes. Generally two passages in a mixer lasting four minutes at a frequency of 12,000 r/m are required. After the second passage and some washings in 0·14 M of NaCl, a suspension of the sediment formed. Drops of this suspension were stained immediately with methylene blue 0·14 M and toluidine blue. Other preparations were stained with Feulgen after fixation according to LA COUR[3].

Normal Chromosomes

It is confirmed in the preparations of normal human chromosomes that during the resting stage, chromosomes exist as single units. In many of them it is easy to distinguish the primary constriction (Fig. 1). The chromosomes partly isolated and partly joined by the ends to each other are seen. There is a sure difference in thickness when a comparison is made between the preparations with methylene blue and those stained with Feulgen (Fig. 2). The chromosomes of normal human leucocytes are rather long and are almost completely devoid of relic coils (Fig. 3). In the majority of cases they are certainly split along the longitudinal axis (Fig. 1). The chromosomes stained with Feulgen take the colour with different intensity in different portions (Fig. 2 and 4). The structural duplicity and the banded appearance are phenomena already pointed out by CLAUDE and MIRSKY for cells of a different kind.

Leukaemic chromosomes

The leukaemic chromosomes observed came from chronic lymphatic and myeloid leukaemia. Owing to the presence of chromosomes of immature cells in these preparations and the theoretic possibility of chromosome breakages caused by the treatment, statistical study of the chromosome set was discarded, but nevertheless certain differences were disclosed between leukaemic and normal chromosomes, that cannot be put down to the above mentioned factors.

As a matter of fact leukaemic chromosomes show heterogeneousness (even in the case of lymphatic leukaemia), which distinguishes them from the normal chromosomes.

Beside the doubly structured chromosomes, the appearance is frequent of single, thin and sinuous chromosomes having a different length (Fig. 6 and 7). In leukaemic chromosomes the relic coils are many and are evident to such an extent that sometimes the chromosome appears to be a knotted strand. Furthermore in the leukaemic preparations, although the same technique

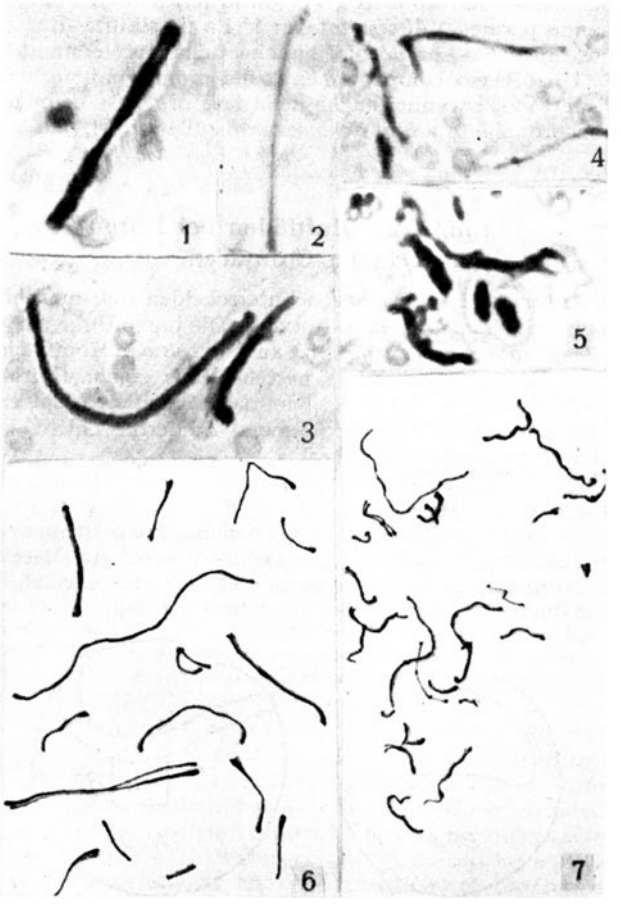

Fig. 1. – Single chromosome of normal white cell with primary constriction and split along the longitudinal axis. Magnification about 2000 diam. Methylene-blue stain.

Fig. 2. – Chromosome of normal white cell. Magnification about 2000 diam. Feulgen stain.

Fig. 3. – Chromosomes of normal white cells. Magnification about 2000 diam. Methylene-blue stain.

Fig. 4. – Chromosomes of leukaemic white cell. Magnification about 2000 diam. Feulgen stain.

Fig. 5. – Contracted chromosomes of leukaemic white cell. Magnification about 2000 diam. Methylene-blue stain.

Fig. 6. – Drawings of normal chromosomes. Magnification about 1500 diam.

Fig. 7. – Drawings of leukaemic chromosomes. Magnification about 1500 diam.

[1] F. FRIMBERGER, Erg. inn. Med. *61*, 680 (1942).
[2] G. WALTER, Klin. Wschr. *19*, 547 (1940).
[3] L. F. LA COUR, Proc. Roy. Soc. Edinburgh, B. *62*, 73 (1944).

was used, short chromosomes are frequent, contracted and overstained (Fig. 5). The aforesaid data are in accordance with what has been already pointed out with other methods in mitosis of leukaemic elements, i.e., the presence of metaphases with overpacked chromosomes.

These observations give evidence of the existence, in the resting stage, of chromosomes similar to the prophasic ones. Furthermore, the comparison between chromosomes of normal and pathological cells brings to light how the latter can be distinguished also by means of abnormalities in the general structure of the chromosome.

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Institute of General Medicine and Institute of Genetics, University of Milan, May 2, 1950.

Riassunto

Sono stati isolati i cromosomi da globuli bianchi umani normali e leucemici. Le osservazioni compiute su materiale colorato a fresco e con la reazione di Feulgen hanno permesso di constatare: 1° La possibilità di riconoscere cromosomi isolati anche nella specie umana; 2° Un diverso comportamento dei cromosomi normali rispetto ai leucemici anche nella fase di riposo cellulare concordamente a quanto osservato sulle mitosi.

Chimärische Haftfäden bei Triton-Unken-Chimären

Triton- und Unkenlarven unterscheiden sich morphologisch im Typus ihrer Haftorgane. Die junge Unkenlarve (*Bombinator pachypus*) besitzt an der *ventralen* Kopffläche zwei Haftscheiben. Diese bestehen aus einem Epithel hochzylindrischer Drüsenzellen, das der äußeren Epidermisschicht angehört. Die innere Epidermisschicht ist nicht besonders differenziert.

Die Haftfäden von Triton dagegen entwickeln sich als stummelförmige Anhänge an der Kopf*seite* und wachsen dann zu ziemlich langen Schläuchen aus. Sie besitzen eine zweischichtige Epidermis, ein axiales Mesenchym (Mesektoderm) und zwischen beiden eine Stützlamelle. Auch hier hat die äußere Epidermisschicht Drüsenzellen.

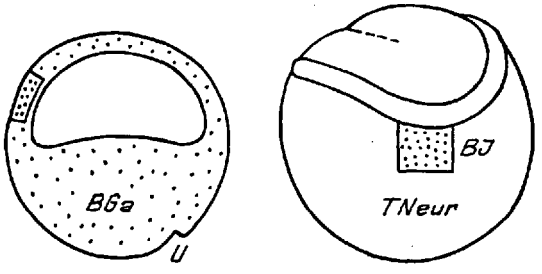

Abb. 1*a* und 1*b*. Operationsschema. *BGa* = junge Bombinatorgastrula. *U* = Urmund. *TNeur* = junge *Neurula* von *Triton alpestris* mit Bombinatorimplantat (*BJ*).

Ein ähnlicher Unterschied in den Haftorganen gilt für andere Anuren- und viele Urodelenlarven. Er bildet eine Ordnungsdifferenz.

Nach dem in Abbildung 1 wiedergegebenen Operationsschema wurde nicht determiniertes Ektoderm der Bombinatorgastrula xenoplastisch in den präsumptiven seitlich-dorsalen Kopfbereich einer Tritonneurula eingepflanzt (*BJ* in Abbildung 1*b*). Das Implantat differenziert sich (ähnlich wie bei HOLTFRETER[1]) zum Teil zu Mesektoderm, zum Teil zu Epidermis. Diese dehnt sich bei der weiteren Entwicklung bis in den Bereich aus, wo bei *Triton* der Haftfaden ausgebildet wird. In der Folge entstehen häufig stummelförmige oder mittellange Haftfäden mit chimärischer *Bo* + *T-Epidermis* und gemischtem axialem *Mesenchym* (Abb. 2, *Hf*). Dicht neben ihnen können auch Bombinatorhaftscheiben auftreten (Abb. 2, *Hdr*). Sie liegen in diesem Fall meistens seitlich.

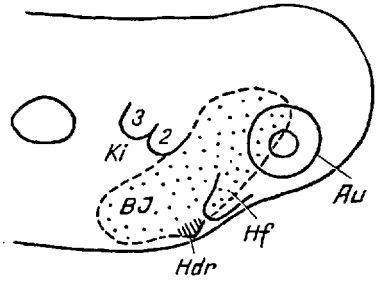

Abb. 2. Chimärischer Haftfaden (*Hf*) und Haftscheibe (*Hdr*) bei einer Tritonlarve, lebend, 4 Tage nach der Operation wie Abb. 1. Es sind nur 2 Kiemenhöcker vorhanden (*Ki 2, 3*). *Au* = Auge, *BJ* = Bombinatorimplantat. Prot. Ch. XVIII 4 T.

Eine gleiche chimärische Entwicklung kommt, allerdings weniger häufig, auch zustande, wenn der Epidermisbereich, wie er in Abbildung 1*b* wiedergegeben ist, zwischen Triton- und Bombinatorneurulen ausgetauscht wird. Es können beide Partner als Wirt dienen. Das Wesentliche ist jeweilen, daß die Grenze zwischen der *Bo*- und *T*-Epidermis *durch* den präsumptiven Haftfadenbereich hindurch geht. Wahrscheinlich ist auch eine zeitliche Koordinierung der beiden sich entwickelnden Materialien von Wichtigkeit.

Bei diesen chimärischen Haftfäden besteht meistens ein Teil oder die ganze *äußere* Epidermisschicht aus Bombinatorzellen. Diese ist oft durchaus regelmäßig, hat aber in den bisherigen Fällen niemals typische Sekretzellen differenziert. Dies war auch nicht zu erwarten, da die Haftfadenbildung erst in einem Zeitpunkt erfolgte, in dem die Haftdrüsendetermination bei Bombinator schon vorüber, im Experiment also «verpaßt» war.

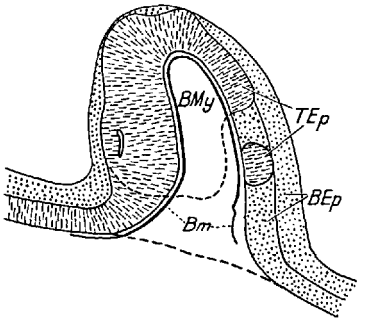

Abb. 3. Stummelförmiger Haftfaden mit chimärischer *T* + *B*-Epidermis; Längsschnitt. Der Hohlraum (*BMy*) ist dicht mit Bombinatormesenchymzellen ausgefüllt. *T*-Gewebe gestrichelt; *B*-Gewebe punktiert. Die gebrochene Linie gibt die Ausdehnung der inneren *B*-Epidermisschicht in den tangentialen Schnitten der einen Seite an. *Bm* = Basalmembran 225fach. (Prot. Ch XV. 4 B. *T-neur* → *B-neur*).

Besonders stark gemischt war eine jüngere Haftfadenanlage, die in Abbildung 3 wiedergegeben ist. Hier besteht ein Sektor der *ganzen* Epidermiswand (*BEp*) aus Bombinatormaterial, und dieser Sektor nimmt in typischer Weise und *offenbar aktiv* an der Haftfadenbildung teil. Die übrige äußere Epidermisschicht (auch Bombinator) ist unregelmäßig.

[1] J. HOLTFRETER, Roux' Arch., *127* (1933).