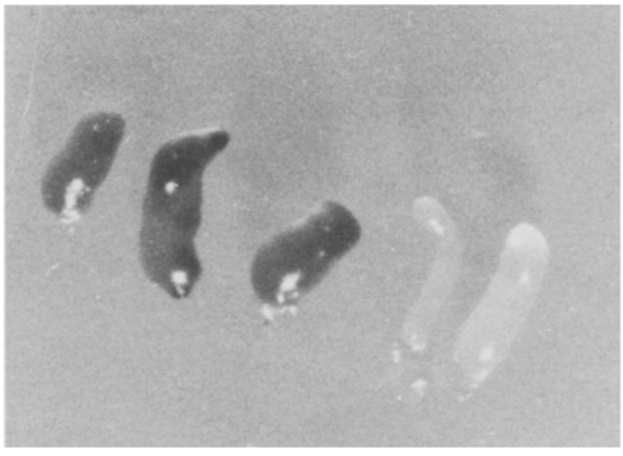

Figure 1. Colored pseudoplasmodia obtained by the simple technique described in the text, observed under a stereo microscope.

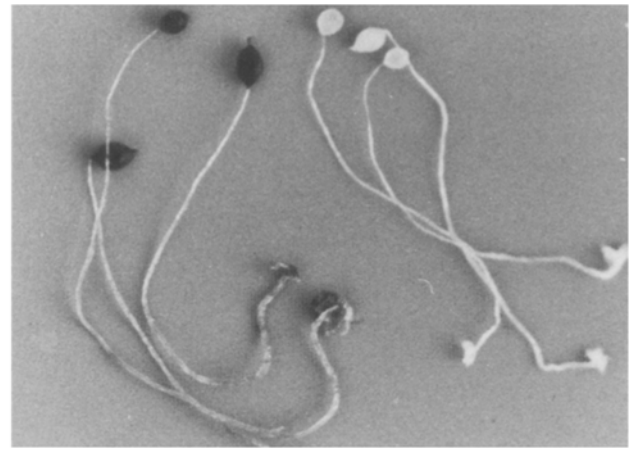

Figure 2. Mature fruiting bodies. The fruiting bodies on the left have a clear red color. The marked cells are stable throughout development. The fruiting bodies on the right have a yellow color (wild type NC-4 has a yellow color).

phosphate buffer pH 6.1) containing the dye neutral red (1 mg/200 ml) at 37 °C for 15 h. The *E. coli* B/r cells were washed 3 times with 10 mM phosphate buffer pH 6.1 by centrifugation, then they were suspended in the same buffer and spores of *D. discoideum* were inoculated. The growth rate of amoebae in cultures with neutral-red treated *E. coli* was similar to that in cultures without dye. After 36 h in suspension culture (at 22 °C, 120 strokes/min) the amoebae were washed with the buffer to remove the stained *E. coli* B/r. Thus we could obtained clearly red-colored amoebae. Except for the suspension culture, the amoebae were incubated on a non-nutrient agar (2%) in the dark. Figure 1 shows clearly red-colored pseudoplasmodia, and others not containing neutral red. The whole of a young pseudoplasmodium is observed to be colored red. However, an old pseudoplasmodium has a strongly stained region; the tip possesses a vacuole which is specific for prestalk cells[7]. Figure 2 shows the fruiting bodies: these were observed to be red-colored all over, but the basal disc regions were especially strongly stained. The present result shows that the cells marked are stable throughout development and the dye (neutral red) is not digested in *D. discoideum*. A dye concentration of 0.1 mg/200 ml in the incubation medium also stained the amoebae, but the cells were only weakly colored.

The technique described here should prove useful not only for studies of cell sorting, but also for studies of photodynamic action and photodynamic mutation in *D. discoideum*.

1 J.T. Bonner, Q. Rev. Biol. *32*, 232 (1957).
2 D. Francis and D.H. O'Day, J. exp. Zool. *176*, 265 (1971).
3 S. Matsukuma and A.J. Durston, J. Embryol. exp. Morph. *50*, 243 (1979).
4 D.H. O'Day and A.J. Durston, J. Embryol. exp. Morph. *47*, 195 (1978).
5 P.A. Farnthworth and L. Wolpert, Nature *231*, 329 (1971).
6 K.B. Raper, J. Elisher Mitchell scient. Soc. *56*, 241 (1940).
7 A.J. Durston and F. Vork, J. Cell Sci. *36*, 261 (1979).

A hyperpolarizing factor is synthetized in a cell at activation of the genetic apparatus

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Summary. A factor, hyperpolarizing the plasmic membrane, is synthetized within a cell at activation of its genetic apparatus.

A special feature of F. Verzár's activities[1] was his attempt to throw light on the metabolic mechanisms of cellular functions. That is why, in memory of this outstanding scientist and remarkable man I am presenting data on the relationships between the activity of genetic apparatus, protein biosynthesis, and electric properties of the plasmic membrane.

The series of experiments performed by our research team revealed the hyperpolarization of protoplasmic membrane evolving in different cells (liver, heart, skeletal muscles) at activation of the genetic apparatus[2-4]. Hormones (sex steroids, insulin, hydrocortisone), regeneration, bloodletting etc. induced the activation of protein biosynthesis. Inhibitors of protein biosynthesis prevented the development of hyperpolarization of cell membranes.

Therefore, the development of hyperpolarization seemed to be linked to the formation of a special factor present at activation of protein biosynthesis. The purpose of this study was to verify this assumption.

Methods. Experiments were performed on male Wistar rats aged 8–10 months. The intracellular membrane potential (MP) of liver cells was measured in vivo. Liver tissue was homogenized in a Ringer solution (1:8 v/v). Cytosolic fraction was taken following centrifugation of postmitochondrial supernatant at 100,000 × g for 100 min in ultracentrifuge L2-50 Beckman (USA).

Effect of donor's liver homogenate upon the membrane potential of recipient's hepatocytes

Experimental conditions	MP's initial value (mV)	15 min	30 min	60 min	120 min	180 min
Homogenate effect after hydrocortisone administration	37.6±0.5	42.7±1.0*	44.2±1.3*	46.4±1.3*	48.6±0.7*	46.0±0.7*
Effect of intact liver homogenate	37.9±0.4	38.3±0.9	38.6±0.9	38.6±0.2	40.2±0.7	41.2±1.7
Effect of actinomycin D upon hyperpolarizing effect of homogenate	37.6±0.4	38.1±0.5	38.3±0.5	39.6±1.2	39.7±1.1	43.0±1.0*
Effect of cycloheximide upon hyperpolarizing effect of homogenate	36.9±0.5	37.3±0.5	37.6±0.2	38.2±0.6	41.0±2.1	41.2±2.3
Effect of boiling upon hyperpolarizing effect of homogenate	36.8±0.3	37.6±0.1	37.5±0.2	37.6±0.2	37.8±0.5	38.0±0.6

* $p < 0.05$

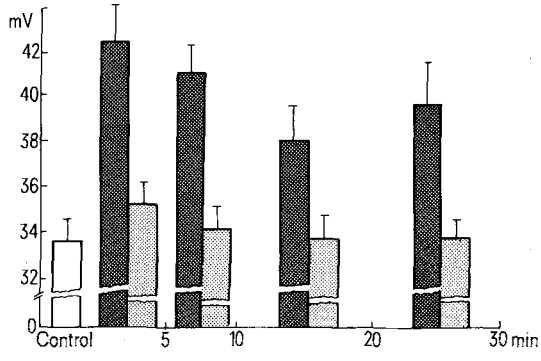

Figure 1. Effect of donor's liver homogenate upon recipient's hepatocyte MP. Black columns - ■, donor after bloodletting; ▨ donor after bloodletting + double administration of cycloheximide (5.0 mg/100 g × 2).

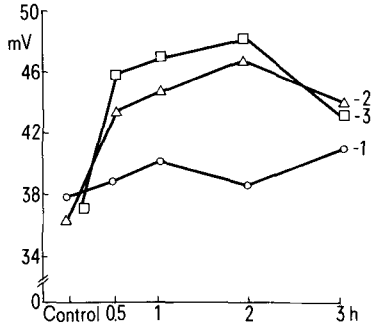

Figure 2. Effect of application of cytosole fraction of liver homogenate of hepatectomized rat donor upon the MP of recipient's liver cells. 1, intact rats; 2, 6 h after hepatectomy; 3, 24 h after hepatectomy.

A special chamber (3 mm in diameter) was fixed onto the liver surface through which the flow of applicating fluid was allowed. Bloodletting was performed from the tail vein (2% of body weight). Partial hepatectomy was performed using the method of Higgins and Anderson[5]. 3.5 mg/100 g hydrocortisone, 15 µg/100 g actinomycin D, and 5 mg/100 g cycloheximide were administered i.p.

Results and discussion. Hydrocortisone administration, bloodletting, and regeneration induced marked hyperpolarization of cell membranes in the liver. 1 h after hydrocortisone administration, the MP increased from 37.9±0.4 to 45.8±1.3 mV. 6 h after bloodletting the MP did not change, while by 24 h it reached 51.8±1.2 mV. 6 h after removal of $^2/_3$ of the liver the MP of liver cells increased from 38.0±0.57 to 48.4±0.53 mV, after 24 h: to 46.1±0.73 mV, after 48 h: to 46.2±0.93 mV.

The hydrocortisone-, bloodletting-, hepatectomy-induced increase of the MP was accompanied by the activation of protein biosynthesis. This was evident from the increase in relative specific radioactivity of RNA and protein and the rise in intensity of renewal of various RNA classes[6,7]. Homogenate of intact rat liver, as well as cytosole fraction produced no significant changes in MP value of recipient rat liver cells (table). Still, the liver homogenate and cytosole, taken at the peak of hydrocortisone-induced hyperpolarization, produced a 8-10 mV increase of the MP in recipient liver cells. Maximal increase of the MP was registered by the 3rd h. Consequently, the hydrocortisone-induced hyperpolarization of protoplasmic membranes may be transferred from one animal to another and is, apparently, related to the formation of a hyperpolarizing factor.

Another series of experiments was designed to study the effect of inhibitors of protein biosynthesis upon the formation of hydrocortisone-induced hyperpolarizing factor. The following inhibitors were used: actinomycin D, capable of blocking DNA-dependent RNA synthesis, and cycloheximide, blocking ribosomal protein synthesis. By blocking protein biosynthesis, actinomycin D and cycloheximide prevent the formation of hyperpolarizing factor. No hyperpolarization was observed in recipient liver cells following the administration of liver homogenate of blockers-pretreated hydrocortisone-treated animals (table).

A hyperpolarizing factor was formed at bloodletting-induced activation of protein biosynthesis as well. As can be seen in figure 1, liver homogenate, prepared 24 h after bloodletting, induced marked hyperpolarization in the recipient. Double administration of cycloheximide (5 mg/100 g×2) after bloodletting blocked the formation of hyperpolarizing factor. Liver homogenate of these animals produced no hyperpolarizin effect (fig. 1).

As is seen in figure 2, the cytosole fraction of liver cells of hepatectomized animals produced marked hyperpolarization of the recipient liver cells.

In a special series of experiments, actinomycin D was administered to recipients whose livers had, thereafter, been given applications of tissue homogenate of hydrocortisone-treated animals. The homogenate produced a hyperpolarizing effect; the MP increased by 8-10 mV. Consequently, the effect of the above hyperpolarizing factor was not mediated by the recipient's system of protein biosynthesis.

Treatment of liver homogenate, obtained after hydrocortisone administration or bloodletting, with 10% trichloroacetic acid did not prevent the hyperpolarizing effect, while a 10-min boiling eliminated this effect (table).

Thus, it can be assumed that the cortisone-, hepatectomy-, and bloodletting-induced activation of protein biosynthesis produces a hyperpolarizing factor in the liver. Inhibition of various links of protein biosynthesis (effect of actinomycin D and cycloheximide) prevents its formation. The effect of this factor upon the recipient cell membrane is not mediated by protein synthesis. Noteworthy is the lag-period of development of hyperpolarization under the homogenate effect - this is shorter than that under the hydrocortisone effect or after bloodletting. This is related to the fact that at

hormone administration and after bloodletting, some time is needed to activate protein synthesis, to synthetize the hyperpolarizing factor, while in case with the homogenate the effect is produced by the 'already available' hyperpolarizing factor. While further investigation is necessary here, it can be assumed, that the hyperpolarizing factor is a peptide. It can be also assumed that, by affecting the state of membrane, the discovered factor adapts the cell metabolism and function to the conditions of intensive protein biosynthesis.

1 See also Experientia *37*, 1039–1059 (1981).
2 V.V. Frolkis, Experientia *28*, 151 (1972).
3 W.W. Frolkis, Mechanismen des Alterns. Berlin 1975.
4 V.V. Frolkis, Experientia *29*, 1492 (1973).
5 G.M. Higgins and R.M. Anderson, Arch. Path. *12*, 186 (1931).
6 V.V. Frolkis, Kh.K. Muradian and N.M. Turaeva, Doklady AN SSSR, *232*, 182 (1977); in Russian.
7 V.V. Frolkis, L.N. Bogatskaya and S.N. Novikova, Fiziologicheski zhurnal *26*, 201 (1980); in Russian.

Allelopathy in spring wheat mixtures[1]

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Summary. In a study with binary mixtures of 3 spring wheat cultivars harmful effects due to allelopathy were observed on root number, root growth and fresh weight of the seedlings.

Allelopathy is generally described as any direct or indirect harmful effect of one plant on another through the production of certain chemical compounds[2,3], although beneficial allelopathic effects through the release of growth hormones have been reported[4]. Roy[5], working with rice mixtures under field conditions, observed that yields from mixtures were higher than the means of the yields from pure stands due to the beneficial effects of allelopathy. In our studies[6–8] with mixtures of spring wheat cultivars under field conditions the mixtures gave a higher yield than the highest yielding cultivar only under adverse weather conditions[7] or under high fertility[6]. The present investigation was therefore taken up to find out whether allelopathy was present in spring wheat mixtures.

Materials and methods. The study was conducted with 3 spring wheat cultivars and their 1:1 binary mixtures. The 3 wheat cultivars differed in plant height and maturity duration. C 306 is a tall cultivar with medium late maturity (155 days), HD 2160 is a dwarf cultivar with early maturity (140 days) and Kalyan Sona is a semi-dwarf cultivar with medium early maturity (150 days). 40 seeds in 4 rows of 10 seeds each were placed in white enamel trays ($38 \times 30 \times 6$ cm). The inter and intra row spacings were 10 and 2.5 cm. In the pure stands all the 40 seeds were of a single cultivar, while in mixtures 20 seeds of each component cultivar were arranged in alternate rows. The trays were watered regularly and kept in an incubator at 30 ± 1 °C for 12 days. The data on root number, root length and fresh weight of seedlings were recorded. All treatments were replicated twice.

Results and discussion. Marked differences between the pure and mixed stands were observed 12 days after sowing (table). The root length, number of roots and fresh weight of seedlings were markedly reduced in all the cultivars when grown in mixtures. As the available space was sufficient for the root growth and the only medium of contact was water, it is likely that the inhibition effect on the roots and fresh weight of seedlings in the mixtures was due to allelopathy. Studies are in progress to identify the chemicals responsible for the observed effects. To our knowledge, this is the first report on allelopathy in spring wheat mixtures, and our objective in publishing this is to initiate similar studies in other laboratories. Studies on allelopathy in crop plants could be important to plant breeders as well as agronomists. In mass selection, allelopathy may play an important role in selecting suitable genotypes. Similarly, allelopathy needs to be studied in mixed and inter-cropping system.

Root length, number of roots and fresh weight of seedlings as affected by pure and mixed stands of wheat cultivars

Treatments	Average root length (cm)			Number of roots (No/seedling)			Fresh weight (mg/seedling)
	3 days	4 days	12 days	3 days	4 days	12 days	12 days
1. HD 2160 (HD)	0.69	1.65	13.10	2.4	2.8	4.7	204
2. Kalyan Sona (K)	0.30	0.75	10.00	0.8	2.7	4.2	179
3. C 306 (C)	0.63	1.14	11.93	0.7	2.2	3.2	191
4. 1:1 HD:K (HD)	0.64	1.69	6.28	2.7	3.0	4.0	116
(K)	0.38	0.80	4.50	0.8	2.3	3.0	95
5. 1:1 HD:C (HD)	0.54	1.50	6.15	2.5	3.0	4.3	141
(C)	0.52	1.42	6.43	1.5	1.9	2.5	82
6. 1:1 K:C (K)	0.24	0.74	6.70	0.8	1.9	3.4	152
(C)	0.58	1.31	8.70	1.1	2.5	3.0	156

1 We thank Director, I.A.R.I. for facilities and encouragement.
2 G. Grummer, in: Die gegenseitige Beeinflussung höherer Pflanzen-Allelopathie, 1st edn, p. 162. Fischer, 1955.
3 E.L. Rice, Allelopathy. Academic Press, New York 1974.
4 H.B. Turkey, Jr, A. Rev. Plant Physiol. *21*, 305 (1970).
5 S.K. Roy, J. Genet. *57*, 137 (1960).
6 R. Prasad and S.N. Sharma, J. agric. Sci., Camb. *94*, 529 (1980).
7 M.R. Reddy and R. Prasad, Biologia Pl. *21*, 85 (1979).
8 S.N. Sharma and R. Prasad, J. agric. Sci., Camb. *90*, 441 (1978).
9 J. Spanring, Savremen. Poljopr., Novi Sad. *14*, 639 (1966).