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EDIDIT: A. B. L. BEZNÁK

HAEC SERIES CONTINUAT EPHEMERIDEM:

MAGYAR
BIOLOGIAI KUTATÓINTÉZET MUNKÁI

ANTEA EDITAM

INSTITUTUM HUNGARICUM AD
BIOLOGIAM PERVESTIGANDAM

TIHANY,

MCMXLVII.

ERRATA

p. III, line 8 read: Sestosoma for Sestoma
p. 19, column 12, 10th line read: reach for rich
p. 20—21, bottom line of Table II. read: collections. (Abbreviated). for callestious. (Abbremated).
p. 21, 10th line from bottom read: $\pm$ 5 dm^2, for $\pm$ 15 dm^2,
p. 40, 5th line from bottom read: habitats for habitribution
p. 69, line 8 read: Picus viridis 1 for Picus viridis 8
p. 69, line 10 read: Muscicapa striata 8 for Muscicapa striata 1
p. 79, bottom line read: Figure 5 p. 81 for Figure 5 p. 79
p. 85, line 24 should read: Dominance groups: $>$ 5% dominant.
p. 89, line 12 read: linnet for Finnet
p. 130, Zeile 10, statt Landesauin: Landesaufn.
p. 134, 5th line from bottom should read: Fasciation of flowers induced etc.
p. 140, 7th line from bottom should read: Fig. 3. Right: fasciated Taraxacum inflorescence etc.
p. 204, line 10 should read: (HAJDU, BEZNÁK 1943; BEZNÁK, HAJDU 1944) etc.
p. 206, following line 4 read: 2 hours each; then 4 extractions with 40-50 ml of Weber II solution,
p. 216, line 14 should read: It is seen in columns 10-13, etc.
p. 233, 2) of Summary should read: The hyperglycaemia caused by coronary occlusion is absent if the branches of the stellate ganglia and of the vagi leading to the heart are cut previous to the ligature.
p. 263, line 10 should read: found that the survival time after the injection of 0.5—0.6 mg/100 g
p. 264, 6th line from bottom read: 401 for 321
p. 268, line 8 should read: -ues compared to ours, though the 350 μg/100 g dose, etc.
p. 273, line 19 should read: normal males (G. I. = 250 μg), etc.
p. 274, for line 16 read: her normal and her HT results. In our experimental groups in these dose ranges we
p. 314, line 7 should read: The correlation coefficient was found to be $r_{wf} = 0.78 \pm 0.77$, and the
p. 330, Zeile 10 statt schröffem: schroffem
p. 332. Zeile 9 statt weise: Weise

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FELELŐS KIADÓ: DR. BEZNÁK ALADÁR IGAZGATÓ.

BARÁTSÁG KÖNYVKIADÓ, LAPTERJESZTŐ ÉS KÖNYVNYOMDAI SZÖVETKEZET,
PÉCS, PERCZEL-UTCA 2
NYOMDÁÉRT FELELŐS: MÉSZÁROS JÓZSEF IGAZGATÓ

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FOREWORD

The year 1945 brought a significant change in the life of the Hungarian Biological Research Institute. The Council of Ministers, at the proposal of *Dr. Count Géza Teleki* Professor at the University of Kolozsvàr and at that time Minister of Education, filled the Institute to capacity by establishing a team of 18 research workers in it. This was intended to serve two purposes: 1.) to enable biological research to be resumed, thus aiding the reconstruction of the country and restoring international scientific collaboration, and 2.) to rescue a considerable portion of the country's biological knowledge.

In 1945 no scientific activity was possible in the laboratories of our universities and other institutions, owing to damage to buildings, lack of heating, light, gas, apparatus, chemicals and experimental animals. The Hungarian Biological Research Institute was about the only institution which had not been impaired by the war, but it stood practically empty, because its personnel — originally a small one — had taken other scientific posts. Here therefore the potentialities for work existed but they remained undeveloped owing to lack of man-power, which in its turn was being wasted in the other laboratories rendered completely unfit for work. Thus by settling this team of research workers in the Hungarian Biological Research Institute the machines of science were manned and the men of science given the tools of work. And this on a site upon which only a few months before fighting had taken place.

In order to serve the other aim, the rescue of as much biological knowledge as possible, it appeared expedient to establish representatives of varied branches of the biological sciences. This moreover has the advantage of mutual stimulation through contact between representatives of not too widely differing disciplines and also the possibility of mutual completion of methodical one-sidedness. Thus the limnologist, the experimental zoologist, the soil-bacteriologist, the animal and plant geneticist, the experimental cytologist, the physiologist, the nutritional physiologist, the bio-chemist and the organic chemist now work side by side in daily contact in this Institute.

During the first year, immediately after the cessation of fighting and amidst the ruins of war and economic troubles, quite extraordinary difficultities had to be overcome. Though every member of the Institute

worked with unparalleled enthusiasm and tenacity during this struggle, it would have been of no avail without the generous help of Hungarian government officials, first of all the Minister of Education, *dr. Dezső Keresztury,* as well as of many foreign institutions and individuals. These acts of human solidarity often procured not only the material necessities for scientific work, but brought back the elevating feeling that we were members of the great brotherhood of men of good will, and gave us moral stimulus beyond all material value.

We dedicate this volume to all those who helped us, as a token of our gratitude.

It is impossible to enumerate them all here, but the most important must be mentioned: The *British Council,* led by *Mr. R. G. C. McNab,* is helping us with gifts of books and subscriptions to periodicals; the *Hungarian Aid in Switzerland* and the *Hoffmann la Roche Company,* on the recommendation of *Professor F. Verzár,* help us with books and valuable chemicals; *Sweden* and the *Swedish scientists* — especially *Professor Y. Zotterman* — with stipends to Sweden and scientific apparatus; and, last but not least, *Professor A. Szent-Györgyi* has helped us by inducing the government at the very start to finance the first steps in reconstruction.

This change in the organization of the Institute brings about a change in the contents of its publication, and this in its turn necessitates certain alteration in the periodical itself. Thus, in future, papers will be published only in congress languages. In consequence, the title of these archives cannot remain Hungarian. In the Latin, most suitable for title purposes, the translation of the present Hungarian title, Archiva Instituti Hungarici ad Biologiam Pervestigandam, is too cumbersome; the short and clear Archiva Biologica Hungarica was therefore chosen. The present volume is therefore the XVII-th as the continuation of the Magyar Biológiai Kutatóintézet Munkái, but under the Latin title it is the first.

We shall strive to keep up the high standard which the Archives attained during the past 20 years under their former editors: Ernest Csiki and Béla Hankó, Frigyes Verzár, Géza Entz, Gyula Méhes, and Sándor Wolsky.

Tihany, December, 1946.

Dr. med. Aladár B. L. Beznák
Director of the Hungarian
Biological Research Institute.

CLADOCERA STUDIES IN LAKE BALATON.

I. MUD-LIVING CLADOCERA AND MUDDY BOTTOM AS ENVIRONMENT.

By: Dr. Olga Sebestyén.

(From the Hungarian Biological Research Institute, Tihany, Lake Balaton.)

With 19 Figures and 1 Table with 4 microphotos.

(Received for publication 26th October 1946.)

INTRODUCTION.

From the results of investigations in the life of the pelagic region as well as of the littoral the conclusion might be drawn that organic detritus plays an important role in the food relations of Lake Balaton. *Dreissena* and *Corophium curvispinum,* species which appeared in the lake about 1931—32 and since have rapidly increased, are consumers of detritus. The significance of the detritus drifts is emphasised by the fact that drift investigations throw light on the mechanism of the origin of the autochtonous detritus in our lake (Entz-Sebestyén, 1946). Thus in plans for further investigation of the life of Lake Balaton more and more place is required for the detritus problem.

Studies in detritus were introduced in our program in the autumn of 1942. However, because of various difficulties caused partly by war conditions as well as by the wide variety of problems involved in the question, the investigations have yet to be completed.

During the qualitative and quantitative analysis of samples collected systematically from detritus-drifts, chitin remnants of Cladocera were frequently found. These remnants consisted mostly of shells not in condition appropriate for indentification, of ephippiums in less number. There were some dead, and a very few living examples. Since species of Cladocera living in the benthic region as well as in the littoral subsist on detritus, it was convenient to combine the detritus

investigations with studies of Cladocera. The aim in this connection is to find the significance of this group in the cycle of matter in our lake, with stress on the detritus problem. The investigations — still in process — are to be carried out by biotops. This might lead to a revision of the Cladocera fauna of Lake Balaton, too, a thing urgently needed, the work at present existing on the subject being DADAY's (1897) enumeration, nearly half a century old.

The limentic region of our lake has a Cladoceran population not rich in species but large in number of individuals, at least during the favourable season *(Leptodora, Diaphanosoma, Daphnia cucullata).* In the cold season the group is represented only by the scanty populations of eurythermous forms such as *Daphnia cucullata* and *Bosmina longirostris.* Water fleas, on account of their seasonal appearance and periodical increase, as well as because of the cyclomorphosis of certain species *(Daphnia cucullata* and perhaps *Bosmina)* take a leading role in the changing character of the zooplankton throughout the year in Lake Balaton (ENTZ-KOTTÁSZ-SEBESTYÉN, 1937). *Leptodora,* the only rapacious water flea of our lake, has been the subject of manifold studies in Tihany (SEBESTYÉN, 1933). However there are many questions still open: the course of seasonal polymorphism of *Daphnia cucullata* and *Bosmina longirostris* has as yet not been thoroughly studied.

Although the main morphological characteristics of our lake shores are not very variable, during the favourable season many „micro-habitats" are formed within the littoral. It seems that the milieu is determined mainly by the presence or by the lack of different species of water-plants *(Hydrocharis, Utricularia, Myriophyllum, Butomus,* etc.) or of filamentous algae. For this reason satisfactory results in the investigation of the littoral with respect to the Cladocera-fauna could be obtained only if collections were made frequently over a couple of years.

In comparison with conditions in the pelagic region and in the littoral, the Cladocera fauna of the muddy bottom seems to be rather limited as to species and scanty as to numbers of individuals.

In what follows an account is given of the investigations made of mud-living Cladocera of Lake Balaton for over one year. To make the description of the milieu and of the life within it more complete, references are made to the results of former studies on the same subjects.

Method.

Mud samples were taken in the open water at about 300—400 metres distance from the eastern shore of Tihany peninsula, in the direction of the main building of the Biological Institute. The water is about 3 m deep here (medium water level). The sediment is mud. Macrovegetation is wanting. This territory belongs to the eprofundal (in LENZ'S sense. 1928). On most occasions — for comparison — hauls were also made from the sediment of nearby places with scanty growth of *Potamogeton perfoliatus*. There the mud is mixed with sand.

The collections were made with the EKMAN-BIRGE apparatus, which device penetrates with ease into the soft mud but always brings up water too. It is not suitable when hard sand is present, however. The hauls — two each time — amounting to about 2×3.5 dm^3 mud, were put into a common metal bucket. After a while the water which gathered on the surface of the mud was filtered carefully through a net of Swiss silk bolting cloth No. 6 (29 threads per 10 mm). The net was rinsed into a porcellain cup and the catch put aside for a while. More water was added to the mud and this was repeated two or three times. The free-swimming individuals were selected from the cup by means of a pipette, while the ones held by the membrane (Chydoridae) were removed by a hair brush, with the help of a hand-loop.

The method described is inadequate when quantitative conditions are involved. For such purposes some other method must be worked out. But because of the poverty of the microfauna of the mud it seems impossible to avoid looking through a considerable amount of mud.

Part of the material was investigated in a live condition, the rest of it was preserved in alcohol and mounted in glycerine.

CONDITION OF EXISTENCE IN THE MUD.

Physical and chemical features. In „Das Leben des Balaton-Sees", by G. ENTZ and O. SEBESTYÉN (1946), a summary is given of our knowledge of the extent and thickness, as well as of the chemical, mineralogical and physical aspects of the mud. This summary is based on the geological investigations carried out by the Balaton Commission of the Hungarian Geographical Society (LÓCZY, 1916). FRANCÉ'S paper on the mud of Lake Balaton (1894) was also taken into consideration. A few later data were also added. For details the reader is referred to the papers by LÓCZY (1916), TREITZ (1911) and EMSZT (1911) and to the appropriate chapters of ENTZ-SEBESTYÉN'S work (pp. 198—207; 341—349).

In the following the main characteristics of the mud are briefly mentioned. The thickness of the sediment covering the bottom of the basin varies from 0 cm to about 16 meters. The upper strata of the

sediment is made of sand and mud, mud being located along the NW shore, sand on the opposite one. The width of the muddy bottom is about 4—5 times that of the sandy one. The zonal arrangements of the mud and sand are caused by wind and wave action. Above the more or less solid mass of mud is a superficial ooze of varying thickness with a semi-liquid consistency („latyak" in the Hungarian language).

Emszt's and Treitz's investigations led to the conclusion that the mud is subaerial in origin, having been brought into the water from the hills of the Balaton Highlands during long ages by northerly winds. Besides these allochtonous ingredients it consists of those originated in the lake itself, namely, products of erosion (Lóczy, p. 627) and biogen lime, as precipitated carbonates of Ca and Mg, being by-products of plant assimilation (Moon, 1934).

The mud when dried is silvery white in colour. It is made up of very fine particles. Chemical analysis (Emszt) shows that it contains much carbonate of calcium and magnesium (± 60%), the SiO_2 content is also high (± 24%), a considerable amount of iron (± 3% Fe_2O_3), aluminium (± 7% Al_2O_3) being also present. The organic content amounts to ± 2.84%. As it contains more lime than the falling dust, part of it must have originated in the mussel shells of the lake, according to Emszt. He did not take into consideration the precipitated lime mentioned above. According to Francé the SiO_2 content comes from valves of Diatoms, but Emszt reports a high percentage in the falling dust too. Pantocsek (1916) studied the Diatom-content of the mud in the surface and deeper strata from a systematical viewpoint; the results did not give a clue as to the value of Diatoms as a mud-producing factor in our lake.

Temperature. Thermic conditions in the mud of Lake Balaton have not been investigated systematically since Sáringer (1906). It must be noted that his measurements were taken 15—20 cm below the surface of the sediment, and no account is given of the method used.

The following is a short summary of Sáringer's findings regarding thermic conditions in the mud of Lake Balaton:

The yearly fluctuation of the mud temperature lies between 0.3°—23.7° C. In comparing the temperature of the mud with that of the bottom water we find that during the cold season (November—February) the temperature of the mud is somewhat higher, during the favourable season (May—August) somewhat lower than that of the bottom water; when warming up (March—April) and cooling down

(October—November) the temperatures of both habitats are inconstant, respectively high and lower temperatures being recorded alternately in the mud and in the bottom water. When the water freezes the mud might cool down to as low as below one centigrade, but as soon as ice is formed its temperature rises slowly but constantly, the rise having, however, a low value. When thaw takes place the temperatures both of mud and bottom water sink, but soon rise somewhat.

In the course of the present studies, due to lack of a suitable instrument (a reversing thermometer by which thermic conditions of the surface of the mud and in any depth desired might be recorded) the temperature of the sediment could not be taken when collections were made. However, to get an idea of the thermic conditions in the mud, a RUTTNER's bottle containing a thermometer was occasionally let down, deep enough to stir up the sediment. The following data (in C°) gained in this way correspond well with SÁRINGER's findings:

		19, VIII, 1946 9.30 A. M.	5, IX, 1946 9.30 A. M.	12, X, 1946 10 A. M. (previous night NW wind)
air		26.5	23.4	12.0
water				
depth m	0	23.2	20.4	11.4
	1	23.1	20.4	11.4
	2	22.9	20.2	11.4
	2.60	22.7	—	11.4
	2.80	—	20.0 turbid water	11.4 turbid water
	3 (bottom)	22.4	19.8 water contains much mud	11.6 water contains much mud

It is known that in our lake there is no thermocline, and the lake is holomictic. Thermic stratification is direct in summer and indirect in winter but always insignificant — if present at all. (ENTZ-SEBESTYÉN, p. 231—239.)

Dissolved oxygen. It is well known that the entire water-body of Lake Balaton is rich in free oxygen (see ENTZ-SEBESTYÉN, p. 250—254). The effect of wave actions reaches to the bottom, and stirs up the loose sediment. Thus a close contact is created between the mud and the water above it. From this it might be concluded that in open water districts the superficial ooze which harbours the benthos is well provided with dissolved oxygen. The smell of the mud is characteristic but not unpleasant.

pH. With SZONNTAG'S electric apparatus for pH investigations the following data were gained on October 26th, 1946:

pH, water	8.10
pH, muddy water	8.15
pH, mud	8.85

Illumination. Because of the turbidity of the Balaton water there exists a stratification of the light in the water. The rate of light-penetration differs during the various seasons and depends on the presence of wind, still or stormy weather. In consequence, light conditions at the bottom vary, and though the lake is shallow they are in general rather unfavourable. Although the presence of holophytic micro-organisms in the mud would be a proof that in the bottom photosynthesis takes place, we have to keep in mind the fact that because of the agitated bottom the algal flora is frequently dislodged and for a while it appears in the higher water-layers, where light is less feeble. (For details of light penetration the reader is referred to ENTZ-SEBESTYÉN'S summary, [1946, pp. 240—244; references]).

Wind and wave action. A wind exceeding the rate of 4 m/sec — a common phenomenon on Lake Balaton — stirs up the sediment (ULLYOTT-KNIGHT, 1938). In such storms fine particles of the bottom become dislocated and are easily moved about. For varying durations they are distributed in the entire mass of the water body of the lake. Intestines of limnetic Cladocera *(Daphnia cucullata)* are filled with mud after stormy weather. The frequent disturbance of the habitat has a strong influence on the life within the mud.

Biological conditions. Food. To determine the nature of the mud from biological viewpoints, investigations were carried out chiefly following NAUMANN'S outline (1925). The sample (one haul by the EKMAN-BIRGE apparatus [with an opening of 15x15 cm = 225 cm^2]) derived from the locality already mentioned, October 7th, 1946.

A) Volume of mud, when wet 3.5 dm^3
Weight „ „ „ „ 4551.4 g
„ „ „ „ dried 1505.0 g

B) Residue of similar quantity sieved through a brass wire sieve with meshes 1 mm^2:

	pieces	dry weight in mg
a) minerogen particles, < 4 mm	22	120.0
b) phytogen particles (tissues), < 10 mm	—	15.3
charcoal, (from reed burning) ± 6 mm	2	63.0

c) zoogen elements,

empty shells of Lithoglyphus	13	770.0
empty shells of Dreissena (halves)	22	1050.0
empty shells of Pisidium (halves)	9	9.6
fragments of mollusca shells, < 7 mm	—	240.0
otolith	4	13.9
Litoglyphus, alive	1	0.053
Chironomidae larvae, alive	7	0.018
Total dry weight of organogen elements:		2161.871 mg

Organogen elements in the residue make up only 1.4 ‰ of total weight.

C) Microscopic investigations:

1) surface of the mud after sedimentation and decantation (Table I. Fig. 1):

a) the sediment is mainly mineral in character;
b) Diatoms, living, and empty valves, some Cyanophyceae;
c) zoogen elements represented by excrement balls (85—100 μ by 25—40 μ), shells of Thecamoeba (very scarce), spicules and their fragments of fresh water sponges (rare). (FRANCÉ reported them from the mud deriving from the littoral where sponges grow abundantly.)

2) From surface of the mud, set aside in the laboratory in a porcellain dish for 48 hours, more algae could be detected, *Ceratium* cysts, fragments of plant tissues and excrement balls were also noted.

3) Investigations immediately after the collection show also that in the sediment finely distributed mineral particles are dominant. Valves of Diatoms are frequent, spicules of sponges rare, coprogenic elements exceptional. (Table I. Fig. 2—3.)

4) To eliminate the lime which constitutes about half the mud, the material was treated with hydrochloric acid. Valves of Diatoms and other organogen elements could thus be detected more clearly. (Table I. Fig. 4).

Summarizing the result of these investigations — which were however made for only a general inquiry — it may be said that *mud deriving from the eprofundal of the lake off the eastern shore of the Tihany peninsula is made up chiefly of unorganic particles. Characteristic elements are valves of Diatoms* both limnetic and benthic, which are quite frequent, and *spicules. Coprogenic elements are scarce.*

As to the *bacteriological conditions* in the mud in comparison with

those in the water, it was found by L. HARANGHY (1941) that the mud is poorer in species but richer in individuals, most of them being those which are common in soil.

The *algal flora* is rather monotonous, consisting mostly of Diatoms. Most of the examples found alive are representatives of benthic species *(Campylodiscus, Cymatopleura, Cymbella, Surirella, Synedra,* etc.), among the empty valves those of limnetic forms are also frequent *(Melosira, Fragilaria, Cyclotella,* etc.). When mud covered with some water is set aside for a while a yellow-brown coat forms on the surface, made of Diatoms. This phenomenon was already observed by FRANCÉ. The amount of Bacillariaceae so appearing varies according to the season and is influenced by weather conditions past and present.

Algae of other groups — mostly of Cyanophyceae — were obtained from mud cultures by E. KOL (1938) (from resting stages?). During the present investigations Cyanophyceae both filamentous *(Lyngbya limnetica* and *L. circumcreta)* and coccoid types *(Gomphosphaeria, Microcystis),* all of them being limnetic forms were but rarely present. Flagellatae both colorless and those with green chromatophores are somewhat more common. *Trachelomonas* is exceedingly scarce. A sample collected in the autumn (September 25, 1946) was especially rich in Eugleninae *(E. Ehrenbergii, E. oxyuris, E. acus, E. intermedia, E. proxima,* (all of them occur in the plankton) and other minute forms; few *Phacus*-species). These formed a green margin in the thin water layer above the mud. A Rhizopod: *Raphidiophrys intermedia* PENARD ($d = 45\ \mu$), which feed on Diatoms and minute Flagellates, was also noted in the same catch. Such an accumulation of green Flagellates in the bottom is however but exceptional. It seems to be in some relation to their occasional decrease from the plankton (see NAGY, 1939).

The chief source of food for the consumers is evidently organic detritus. This is partly subaerial in origin (TREITZ), partly planktogen, most of it very likely deriving from the macrovegetation of the littoral. Because of the low specific weight of plant debris and of the restlessness of the water-body of our lake fine particles of detritus from the littoral can be carried away easily into the open water, and for the same reasons can remain floating there for a long time and serve as food for members of the zooplankton (Cladocera, Rotatoria, young fish). A minor part of it, however, sinks to the bottom and mixes there with the mud. If mud is filtered through a net of silk bolting cloth No. 6, a mass of brownish coloured detritus remains, with particles very

little larger than those which fall through the meshes. The more finely disintegrated particles could not be detected thus. It seems that food conditions in the mud are less favourable than for pelagic and littoral species of Cladocera. The intestines of mud-living species are filled with mud. When this is treated with hydrochloric acid a mass of fine particles is left, this being very likely residue of organic detritus.

The *micro-fauna* is rather static, limited in species and few in number. In the catches *Ectinosoma Edwardsii,* a minute Harpacticida, of brown colour, occurred the most frequently. Cyclopidae are fewer in number. A few minute Turbellarians, *Micronecta,* Hydracarina and Chironomidae-larvae were always present; here and there a *Lithoglyphus.*

LIST OF CLADOCERA FOUND IN THE MUD SAMPLES.

1. *Macrothrix laticornis* (JURINE) 1820.

A mud-living form. Occurs the year round, being somewhat more frequent in winter. Colourless, sometimes with a yellowish tinge; valves opaque, loaded with mud particles, concealing structure. Females with two eggs or embryos in the brood case. Neither ephippial females nor males were found. Length ♀ ± 450 μ.

Individuals were collected in the margin of the lake after storm among floating detritus particles. These have more colour than those found in mud.

2. *Alona quadrangularis* (O. F. MÜLLER) 1885.

Occurs throughout the favourable season; from November till May but sporadically. It has been found together with *Alona affinis,* being less frequent than the latter. Neither ephippial females nor males were found. Valves transparent, colour different shades of yellow, body sometimes reddish. Length ♀ 520—720 μ.

3. *Alona affinis* (LEYDIG) 1860.

Occurs during the whole year, common in mud and bottom with scanty *Potamogeton perfoliatus* growth. Most individuals were caught in early winter. Colour: various shades of yellow. Males were not found in the mud. Length ♀ 730—900 μ.

It is common among the marginal weeds of the lake also, where it appears as a monocyclical, and warm stenothermous form.

4. *Rhynchotalona rostrata* (KOCH) 1841.

Only few individuals were caught. Males in October. Length ♀ ± 450 μ.

Common in weedy margin of the lake, where it is also a monocyclical warm stenothermous form.

5. *Leydigia acanthocercoides* (Fischer) 1854.

A typical mud-living form, monocyclic, warm stenothermous.

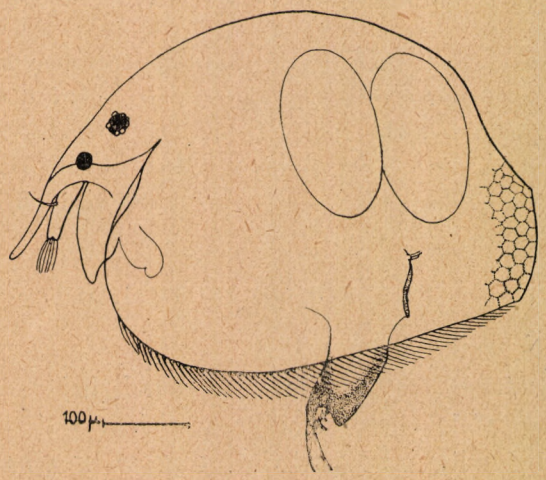


Fig. 1. *Pleuroxus balatonicus* Daday, ♀, from mud. Tihany, Aug. 8th, 1945.

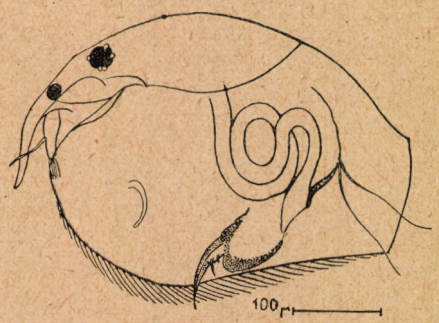
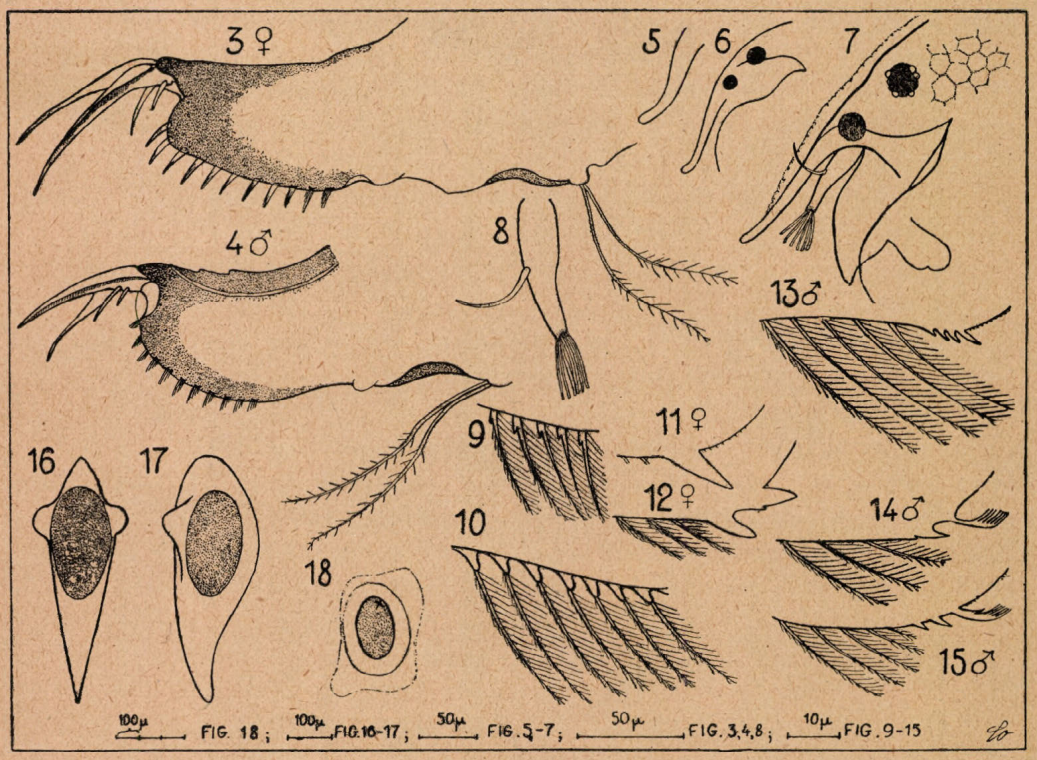


Fig. 2. *Pleuroxus balatonicus* Daday, ♂, from weedy margin of the bay Kis-öböl, Tihany, Oct 18th, 1944.

Ephippial females and males from early August on. Valves yellow; transparent; striated, reticulated, sometimes tuberculated, the reticules often with very fine striae. Claws with delicate pecten; basal spines wanting. Length ♀ 600—800, ♂ ± 560 μ. During the favourable season it was collected, not regularly, from the littoral among weeds.

It seems that individuals with very fine striae on the valves were described by DADAY as Alona Balatonica (1888, 1897). A similar structure of the shell was noted occasionally in other species.



Figs. 3—18. *Pleuroxus balatonicus* D a d a y figs. 3—4, postabdomen of female and male specimens, collected from mud; figs. 5—7, rostrum forms, ♀ fig. 8, antennula, ♀; figs. 9—10, parts of ventral margin from the infero-anterior region of the valve, showing the long, close-set, plumose setae with basic furca, from different views; figs. 11—15, different form of infero-posteal angle, showing teeth of various number, size and shape; also plumose setae, characteristic of the infero-posterior region of the valve; figs. 16—17, ephippium from different view, showing lateral buckles; fig. 18, ephippium as seen from the side, X place of Buckle.

6. *Pleuroxus balatonicus* DADAY 1885.

During the favourable season a *Pleuroxus* species was found in the mud, and the weedy margin of the lake. It seems to be identical with DADAY's *Pleuroxus balatonicus* 1885. (Figs. 1—18)

It is a monocyclical, warm stenothermous form, missing during

most of the year (XII—VI). Males occur in November. It belongs to the short, high-arched form of the genus, having a pointed rostrum curved slightly forwards. (WARD & WHIPPLE p. 726). (Figs. 1, 2, 5—7). Teeth at the infero-posteal angle of the valves are in most cases missing in the female but seem always to be present in the male. (Figs. 11—15). Valves are reticulated, sometimes with tubercules, and are covered with a colourless, adhesive substance, into which particles of mud stick (fig. 7); opaque, colourless, end of post-abdomen, claws and mandibles smoky-gray. Valves with buckles on the sides, which are present on the ephippium too (figs. 16—18).

Post-abdomen of the male differs definitely from any other species of the genus, being very similar to that of the female (figs. 3—4). The species closely resembles *Pleuroxus aduncus* (JURINE) 1820. Length ♀ 550—600 μ.

Individuals found in the littoral among weeds are larger in size, yellow, and have prominant fornices.

7. *Monospilus dispar* (KOCH) 1861.

Mud-living form, monocyclic (?), warm stenothermic. Colour: yellow; transparent. Males from August on. Length ♀ 400—420 μ. Common in the littoral during early spring and late fall.

Occasionally samples of pelagic Cladocera were also found in the catche-

GENERAL REMARKS.

As is seen, during the present investigation seven species of Cladocera were found which, with the exception of *Macrothrix laticornis*, all belong to the family Chydoridae. Though the body-structure of *Macrothrix* seems to be more delicate than any of the representatives of the Chydoridae, with the exception perhaps of *Pleuroxus*, the mode of life of all these mud-living species seems to be similar. They live in the superficial ooze and subsist on mud, utilizing its scanty organic content, which is organic detritus. They do swim, but in general rather scramble about within the surface-layer of the mud. Their organs of locomotion are the second antennae which are rather short, except in the *Macrothrix*. The post-abdomen is also an efficient aid to locomotion (WARD-WHIPRLE p. 679). Their legs are especially adjusted for collecting food from a somewhat solid mass and for pushing the particles towards the oral opening. The first pair of legs is a very complicated tool, equipped with numerous hairs, hooks and spines of va-

rious sizes, shapes, curvature and armature. The structure and action of the legs, in comparison with those species having different food habits (Daphnidae) must be the object of special studies.

It seems of special interest that though these seven species are harboured in the same milieu the surface of their shells is very different. In the case of *Macrothrix* and *Pleuroxus* the valves are coated by some colourless adhesive substance in which minute particles stick. The surface of the shells of the rest of the Chydoridae found do not moisten. (This quality is expressed in German by „unbenetzbar", in French „non-mouillable". (see BREHM 1930, 2—5).

The species having such shells are easily caught in the surface membrane of the water. The quality does not entirely protect the shell against the attachment of epizoa. They were found during the summer months on *Macrothrix, Pleuroxus, Leydigia* and *Monospilus.* The epizoa when belonging to the group of Peritricha were attached in most cases on the inside of the valves along its hind margin. This reminds one of the location of a clump of *Dreissena* on the free part of the shells of Unionidae. On *Monospilus* a tube-living Rotifer *(Collotheca)* was also found, settled on the outside of the shell. The shell of *Monospilus* not being cast off in moulting seems suitable for the attachments of a sessile organism having such a solid case as this Rotifer.

It seems that *the role the Cladocera* harboured in the mud have *in the cycle of matter in Lake Balaton* has something to do with their mode of life and their minute size. They utilize the organic matter sparsely present in the mud of our lake chiefly in the form of fine particles of organic detritus, so that this matter is saved for a time from immediate decay, and in turn they convert it into live organic matter again, edible for other mud consumers of larger size, or perhaps for carnivorous fresh water animals. On the other hand, by their continuous feeding a considerable amount of excrement is produced, by which the structure of the mud is affected, and the organic particles become more accessible to the activities of bacteria. This however has not much significance in an agitated bottom.

Most members of the Cladocera fauna of the mud are harboured by the weedy margin of the lake too. However there are some differences as to habit *(Pleuroxus, Macrothrix)* and life-cycle. *Rhynchotalona, Leydigia, Pleuroxus* and *Monospilus* are monocyclic and warm stenothermous in both habitats. Although neither males nor ephippial females of *Alona quadrangularis* were found, the life-cycle of this spe-

cies cannot be determined as yet, because of its sporadic appearance. *Macrothrix* and *Alona affinis* are eurythermous perhaps acyclic in the mud, while warm stenothermous and monocyclic in the littoral. The diversity in the life-cycle and in demand for temperature may find explanation in that the lack of seasonal changes, especially of dominant force (cyclic life of rooted vegetation, effect of the change of water

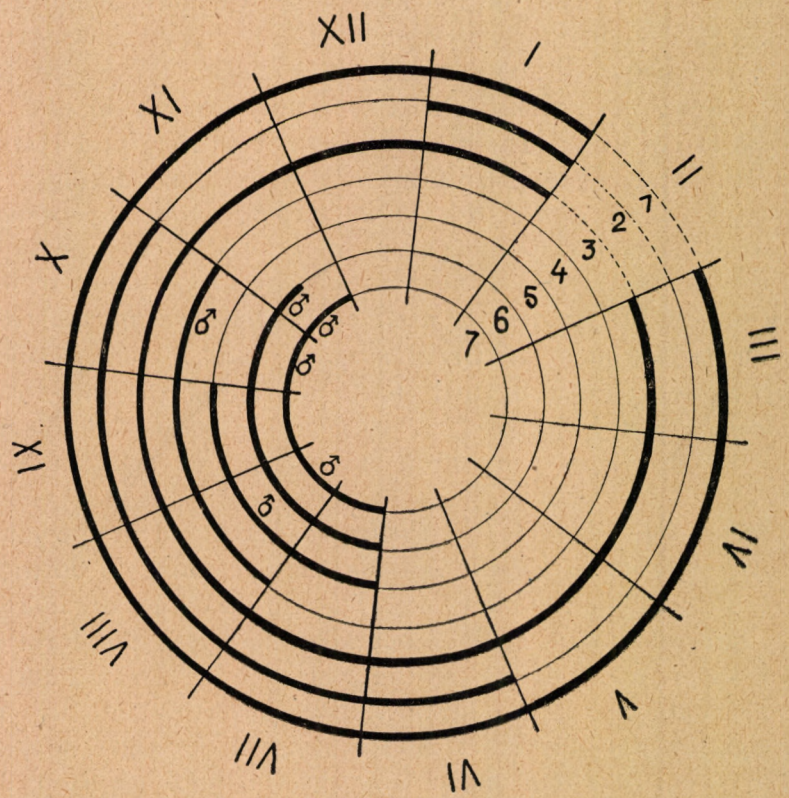


Fig. 19. Diagram showing the presence of Cladocera and the appearance of males in muddy bottom throughout the year, June 1945 — October 1946.
1. *Macrothrix laticornis;* 2. *Alona quadrangularis;* 3. *Alona affinis;* 4. *Rhynchotalona rostrata;* 5. *Leydigia acanthocercoides;* 6. *Pleuroxus balatonicus.* 7. *Monospilus dispar.*

level) makes conditions more uniform throughout the year in the mud. The frequency and scale of mechanical disturbance is less in the bottom than in the littoral. (Fig. 19)

When weighing the factors of the milieu as to their role in in-

fluencing the quality and quantity of the micro-fauna of the mud, we come to the conclusion that there are two factors which are principally responsible for its paucity: scarcity of food, and the frequent mechanical disturbance of the habitat. These factors, in addition to a third one: the lack of bottom vegetation, were pointed out by H. P. Moon when studying the bottom fauna of our lake. (It must be noted that in Moon's investigations only those members of the fauna were considered which could be caught in a sieve of meshes 1 mm^2.

The micro-fauna including Cladocera is but a fragment of the association of bottom-organisms. Further knowledge of the micro-flora and micro-fauna — including quantitative items — are needed to unfold the structure of the benthos, to disclose the manifold relations of its members to each other, to their own habitat, and to the life of other biotops of the lake.

SUMMARY.

Investigations extending over one year were made regarding the life of the Cladocera in the mud of Lake Balaton. The muddy bottom as their habitat was also studied.

Mud deriving from the eprofundal is made up mostly of mineral particles, chiefly lime. Characteristic elements are valves of Diatoms and spicules of fresh-water sponges. Excrement balls are scarce.

Conditions of existence of the mud — with the exception of the wind and wave action — are rather monotonous throughout the year.

The micro-flora consists of Diatoms, Flagellates and Cyanophyceae, among which limnetic forms also occur.

The Cladocera fauna of the mud (eprofundal) is more limited than that of the pelagic region and of the littoral. From the seven species found (one Macrothricidae, six Chydoridae) two (perhaps three) are present the year round of which neither males nor ephippial females were found. Representatives of same species when living in the littoral are warm stenothermous and monocyclic *(Macrothrix, Alona affinis)*. All Cladocera consume mud, utilizing chiefly its detritus content.

For the paucity of the Cladoceran-fauna (and for the micro-fauna in general) two factors might be responsible: the poor trophic conditions of the mud and the agitated bottom.

The presence of pelagic organisms in the benthos seems to be peculiar to Lake Balaton, demonstrating the close contact between epilimnion and bottom in this lake.

Grateful acknowledgment is made to Mrs. J. Thompson Vass for help in the English translation.

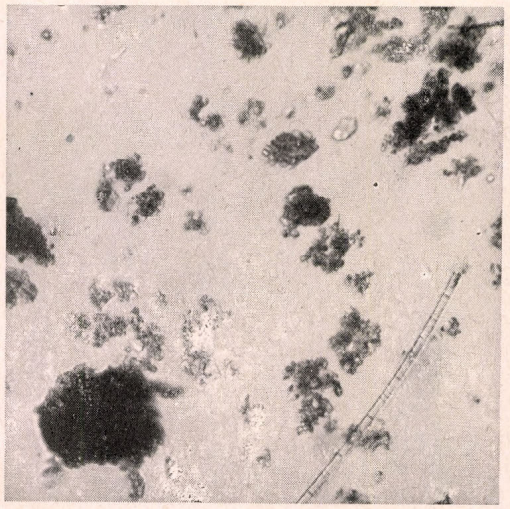
REFERENCES.

Brehm, V. (1930). *Einführung in die Limnologie. Biol. Stud. Bücher* **10**, Berlin: J. Springer.
Daday, E. (1884—85). *Math. Naturwiss. Ber. Ungarn.* **3**, 179.
Daday, E. (1888). *Crustacea Cladocera Faunae Hungaricae. Budapest: Kir. Magy. Term. tud. Társ.*
Daday, E. (1897). *Res. Wiss. Erforsch. Balatonsees, Wien,* **2.** 1. Sect. 9.
Emszt, K. (1911). *Res. Wiss. Erforsch. Balatonsees, Wien.* **1.** 1. Anh. 7.
Entz, G., Kottász, J. & Sebestyén, O. (1937). *Magy. Biol. Kut. Munk.* **9.** 1.
Entz, G. & Sebestyén, O. (1946). *Magyar Biol. Kut. Munk.* **16.** 2.
Francé, R. (1894). *Földtani Közl. Budapest,* **24,** 142.
Haranghy, L. (1941). *Magyar Biol. Kut. Munk.* **13,** 57.
Kol, E. (1938). *Magyar Biol. Kut. Munk.* **10,** 161.
Lenz, Fr. (1928). *Einführung in die Biol. d. Süsswasserseen. Biol. Stud. Bücher* **9.** Berlin: J. Springer.
Lóczy, L. (1916). *Res. Wiss. Erforsch. Balatonsees. Wien.* **1.** 1. Sect. 1.
Moon, H. Ph. (1934). *Magyar Biol. Kut. Munk.* **7,** 170.
Nagy, I. (1939). *Magyar Biol. Kut. Munk.* **11,** 251.
Naumann, E. (1925). *Abderhalden's Handbuch Biol. Arbeitsmeth.* **9,** 2, 1. 103.
Pantocsek, J. (1916). *Res. Wiss. Erforsch. Balatonsees, Wien.* **1.** 1. Sect. 1. 648.
Saringer, J. (1906). *Res. Wiss. Erforsch. Balatonsees, Wien.* **1.** 5. Sect. 1.
Sebestyén, O. (1933). *Magyar Biol. Kut. Munk.* **6,** 104.
Treitz, P. (1911). *Res. Wiss. Erforsch. Balatonsees, Wien,* **1.** 1. Anh. 6.
Ullyott, Ph. & Knight F. C. E (1938). *Magyar. Biol. Kut. Munk.* **10,** 254.
Ward, H. B. & Whipple, G. Ch. (1918). *Fresh-water Biology. New York: Wiley and Sons.*

EXPLANATION OF FIGURES IN TABLE I.

Figs. 1—4. Microphotos of slides prepared from mud deriving from open water district off the eastern shore of the Tihany peninsula. October 7. 1946; magnified 210.4 ×. Fig. 1, mud from the surface of test-tube after sedimentation, showing mineral particles, excrement ball and *Melosira*. In water. Fig. 2, mud in natural condition, showing mineral particles and spicula of fresh-water sponge. In water. Fig. 3, mud in natural condition showing mineral particles. Diatoms and fragment of mollusca shell (?). Mounted in Canada balsam. Fig. 4, slide treated with hydrochloric acid and mounted in Canada balsam, showing mineral particles other than lime and *Melosira*.

Table I.



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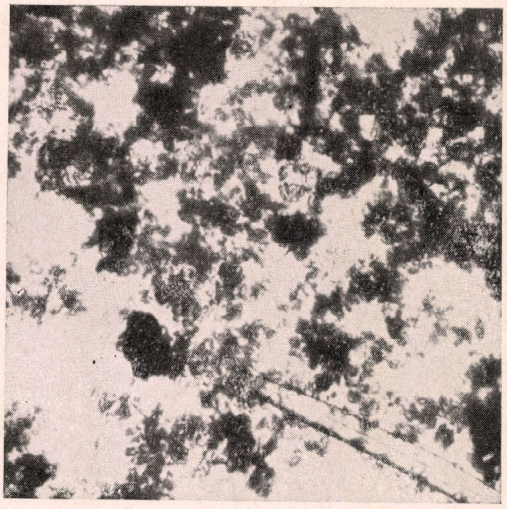

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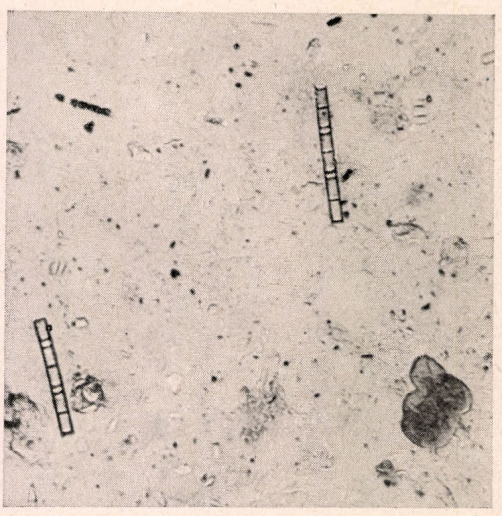

4

QUALITATIVE AND QUANTITATIVE STUDIES IN THE COATINGS OF POTAMOGETON PERFOLIATUS AND MYRIOPHYLLUM SPICATUM IN LAKE BALATON.

By: Béla Entz.

(From the Hungarian Bioligical Research Institute, Tihany, Lake Balaton.)

With 3 Tables and 5 Figures in the text.

(Received for publication 30th November 1946.)

In the autumn of 1946 investigations were made of the coatings on the larger aquatic vegetation of Lake Balaton. Under "coatings" („Aufwuchs") we understand, according to Ruttner „. . . alle einer festen Unterlage anhaftenden aber (im Gegensatz zu dem im Boden wurzelnden Pflanzen oder gewissen Parasiten) n i c h t in diese eindringenden Organismen." (Ruttner, 1940, p. 118.) (Lenz, 1928, p. 115.) In what follows the expression "coating" will be used in its wider sense. We are to understand not only the living organisms covering the surface of the plant, but also the detritus deposited among them, as well as particles of mud, precipitated lime, etc. I made my investigations on the most common submerged water plants, *Potamogeton perfoliatus* and *Myriophyllum spicatum*. These form growths of greater or smaller extent. (Entz-Sebestyén, 1946, p. 331—337.) Investigations in reed-coatings in Lake Balaton carried on throughout the entire year were made by A. Meschkat (1934).

The present investigations of *Potamogeton* and *Myriophyllum* growths were pursued in small areas in the neighbourhood of the Tihany Peninsula. I was interested in the coating structure, its fauna and the relations of the organisms to one another. My aim was to inquire into the differences which are to be seen in the coatings, as to habitat (bays, open water), plant species *(Potamogeton, Myriophyllum)* and different strata.

I collected the plants at Tihany in the neighbourhood of the Biolog-

ical Institute (Figure 1.). The two *Myriophyllum* growths extended over scarcely more than a few square meters. The one (I) ran parallel to the north shore of the Kis-öböl (a small bay); the other (IV) close to a pipeline in the open water. Both of them grew in thick and homogeneous groups several metres in length and a metre and a half wide.

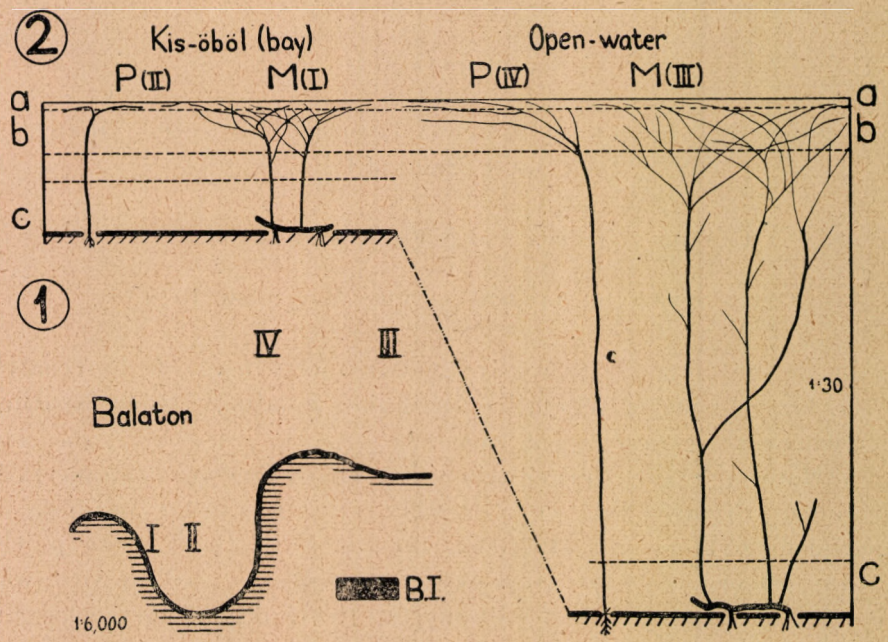

Fig. 1.

Places of collecting. I. Myriophyllum growth running parallel to the shore of the Kis-öböl. Water depth 50 cm. II. Potamogeton growth in the midst of the bay. Water depth 50 cm. III. Potamogeton growth in the open water, cca. 100 m off the shore. Water depth 180 cm. IV. Myriophyllum growth in the open water cca. 80 m off the shore. Water depth 180 cm. B. I. = Biological Institute.

Fig. 2.

Habit of Potamogeton and Myriophyllum plants in the bay and in the open water. P = Potamogeton, M = Myriophyllum. I—IV. places of collecting (see Fig. 1.) a, b, c strata.

The two *Potamogeton* growths were not to be found in such homogeneous groups as the *Myriophyllum*. They were more widely dispersed, and here and there mixed with *Myriophyllum*. As I carried out my investigations on these small plant groups (characteristic, however,

TABLE I.

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
VIII.											
30	I	a	I	M	16	8	1.0	21.0	24.0	Gentle	
„	„	b	I	„	21	9	1.2	„		S-wind	
„	„	c	I	„	48	8	2.0	—			
IX.											
4	II	a	II	P	16	14	1.2	21.0	27.0	Calm	Potamogeton at its full development
„	„	b	II	„	27	20	1.8	„			
„	„	c	II	„	20	12	3.0	—			
IX.											
10	III	b	I	M	86	53	6.8	22.0	22.0	Breeze	Collection after a storm
„	„	c_1	I	„	64	15	3.4	„			
„	„	c_2	I	„	180	50	10.6	—			
IX.											
13	IV	a	III	P	35	13	3.4	20.8	23.2		
„	„	b	III	„	66	34	4.5	„		Calm	Potamogeton very much decayed
„	„	c	III	„	37	5	1.6	—			
„	„	d	III	„	43	6	1.9	—			
IX.											
22	V	a	IV	M	75	34	4.4	17.5	19.2	Gentle	
„	„	b	IV	„	478	127	20.0	„		N-wind	
„	„	c	IV	„	57	12	1.6	—			
IX.											
27	VI	b	II	P	35	17	2.0	20.0	21.0	Calm	
„	„	c	II	„	23	11	2.8	—			
X. 1.											
9h	VII	b	III	P	115	76	9.6	17.6	15.5	Gentle	
„	„	c	III	„	75	7	2.5	—		S-wind	
X. 1.											Only few plants of Potamogeton rich the surface.
14h	VIII	b	III	P	131	67	7.6	18.2	20.2	Calm	
„	„	c	III	„	48	5	1.7	—			
X. 1.											
20h	IX	b	III	P	76	45	7.1	18.0	13.2	Calm	
„	„	c	III	„	65	11	2.3	—			
X.											
8	X	b	IV	M	198	96	12.5	13.2	11.2	N-wind	Previously windy days.
„	„	c	IV	„	145	30	4.7	—			

Circumstances at collecting. 1. Data. 2. Number of the collection. 3. Stratum. 4. Locality. (I—II = Kis-öböl, III—IV = open water) 5. P = Potamogeton, M = Myriophyllum. 6. Stem-length in cm. 7. Number of leaves (Potamogeton) resp. number of leaf-groups (Myriophyllum) 8. Surface of samples in dm^2. 9. Water temperature in C°. 10. Air temperature in C°. 11. Wind. 12. N. B.

of the Balaton) my conclusions cannot be said to be valid for growths of great extent.

The investigations took place between August 30th and October 8th, 1946. During this time dry, temperate weather reigned and neither the water-level nor the temperature showed any appreciable variation. In general the wind velocity remained under 3 m/sec. During the collecting periods the greatest wind velocity was 4.6 m/sec (on one occasion, September 21st) but that also caused no large waves in the regions where the collections were made, as they were in wind-protected places. Thus neither the water plants nor their coatings suffered any damage by wave action. The slow, seasonal, normal decay of the *Potamogeton* was to be observed in its usual autumn progress. (Table I.)

Meschkat, in his quantitative studies, always used 20 cm pieces of reed stem (Meschkat, 1934, p. 446). They all had about the same surface (± 0.63 dm^2). I used not only the stems but also the leaves of the *Potamogeton* and *Myriophyllum* in my studies. It is almost impossible to choose samples with equal surfaces because their habit differs from that of the reed, they differ from each other *(Potamogeton* and *Myriophyllum),* and also differ in different plants of the same species (size and number of leaves, growth of stem, etc.). Therefore in my collecting I took into consideration only the depth in which the samples were taken. Both plants grow perpendicularly erect. When their length exceeds the depth of the water they bend and lie parallel with the surface. I took my specimens in general from 3 strata: 1.) From immediately below the surface, from that part of the plant which lies parallel with the surface of the water (stratum "a"). 2.) The second specimens were taken from surface level to about 20 cm lower (stratum "b"). 3.) Immediately above the bottom, 20 cm long stems (stratum "c"). 4.) In open water one specimen was taken from a depth of 1 m (stratum "d"). (See Figure 2.)

The complete portion of the plant was collected, in whatever stratum, without regard to the lenght of the stem, the number of branches or leaves. The samples were all considered separately, in respect to strata, locality and plant species, in a way to be described below.

The collections were made from a boat. Wherever possible plants with intact leaves were chosen. In shallow water the whole plants were carefully gathered at their roots. The pieces selected, as described above, were cut and put into a vessel containing running water. In deep water the specimens from the different strata were taken without regard for its being the same plant.

TABLE II.

A B	Myriophyllum spicatum											Potamogeton perfoliatus														
	Ia	Ib	Ic	IIIb	$IIIc_1$	$IIIc_2$	Va	Vb	Vc	Xb	Xc	IIa	IIb	IIc	IVa	IVb	IVc	IVd	VIb	VIc	VIIb	VIIc	VIIIb	VIIIc	IXb	IXc	Total
1. Punctodora sp.	567	336	1071	49	374	230	114	80	150	231	229	179	308	88	9	69	48	103	1036	1211	730	2523	649	15	6	104	10509
2. Nitocra inuber	240	589	297	120	31	19	1	6	8	20	117	163	218	96	17	55	24	210	329	407	91	278	49	8	49	256	3698
3. Corophium curvispinum	0	40	66	2	6	3	25	83	426	47	350	26	250	46	6	148	162	190	623	205	67	148	97	74	18	124	3232
4. Chironomida larvae	44	305	47	215	112	48	57	106	44	50	81	121	205	42	67	29	16	72	340	43	50	111	22	73	35	521	2856
5. Rotatoria	604	300	192	101	32	4	110	41	20	28	70	594	134	31	61	107	0	10	19	7	77	0	8	37	31	63	2681
6. Dreissena polymorpha	0	11	263	0	0	0	20	51	106	63	243	5	39	26	2	102	146	463	3	65	25	173	54	341	17	71	2289
7. Nais ssp.	13	150	32	7	19	15	35	7	17	38	11	26	98	29	7	28	16	50	149	91	115	65	69	301	21	99	1508
8. Polycentropida larvae	6	0	41	0	8	10	0	2	8	4	12	0	42	25	0	24	237	184	3	36	7	108	15	119	73	219	1183
9. Stylaria lacustris	19	300	116	59	4	23	3	8	7	23	12	37	84	262	0	1	36	10	30	58	0	33	2	11	3	5	1146
10. Sida crystallina	13	0	0	0	0	0	0	5	0	3	3	263	7	0	31	277	0	10	13	4	101	43	34	7	149	27	990
11. Chaetogaster langi	0	5	22	1	12	5	17	2	0	5	8	0	53	15	13	1	36	40	9	164	16	0	14	63	17	35	573
12. Turbellaria	82	16	19	3	8	7	0	0	8	5	0	21	14	80	6	0	0	7	0	9	1	3	2	0	5	11	307
13. Aeolosoma quaternarium	38	0	6	0	13	3	0	0	28	5	5	0	21	4	0	0	4	13	0	23	0	0	2	7	1	27	200
14. Acroperus harpae	145	0	0	6	1	0	0	0	0	3	1	21	3	0	0	4	0	0	0	0	7	8	0	0	0	0	199
15. Cyclops serrulatus	19	21	0	6	0	0	0	0	0	26	4	0	0	2	0	16	0	0	0	20	3	13	6	0	15	27	178
16. Hydra sp.	0	0	0	0	2	0	0	0	0	1	41	0	0	19	22	3	0	7	0	0	1	0	0	0	0	0	96
17. Ephemerida larvae	0	0	3	0	0	1	0	0	0	0	0	0	4	29	0	0	0	0	0	7	0	0	0	0	0	0	44
18. Dorylaimus sp.	0	5	3	0	4	0	0	0	0	7	5	0	4	0	0	0	0	0	0	0	0	0	2	0	0	3	33
19. Alona sp.	0	0	9	3	0	0	0	1	0	1	0	0	4	0	0	3	0	0	0	2	0	0	0	0	4	0	27
20. Helobdella sp. cocon	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
21. Hydracarina	0	0	6	0	0	0	0	0	0	0	1	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	13
22. Daphnia cucullata	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	11
23. Diaptomus gracilis	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	1	3	1	0	1	0	10
24. Chaetogaster diaphanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	1	0	0	0	0	0	10
25. Ectinosoma Edwardsii	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0	8
26. Notonecta sp.	0	0	0	0	2	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
27. Limnaea sp.	0	0	0	0	1	2	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	6
28. Lepidoptera larvae	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	5
29. Agraylea sp.	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
30. Herpobdella	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
31. Unionida (juv.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
32. Ostracoda	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total	1790	2078	2202	572	631	371	385	392	822	568	1213	1456	1494	796	241	872	725	1372	2563	2354	1298	3509	1027	1056	445	1612	31844

Results of collections

A = species or other syst. group. B = Number and depth of collection. Total number of animals found in callestious. (Abbremated.) (The whole material calculated to the surface of 6.3 dm^2.)

Examination took place immediately after the collecting, while the material was in a live condition. The water was filtered through a net of Swiss silk bolting cloth No. 6 (29 threads/10 mm), which let pass only the Protozoa and the Nematoda of the size of *Punctodora*. From the filtrate, stirring constantly, a sample was taken with a piston pipette of a known volume (5 cm^3), and the *Punctodora* in them counted; the number obtained was used in calculating the number of organisms *(Punctodora)* in the entire filtrate. After this all the organisms remaining in the filter were counted in a little water in a Petri dish marked in sq. cm, with the aid of a binocular praep. microscope, enlargement 20x. After this, by means of several rinsings and a final delicate rubbing, the coatings were entirely removed from the plant and the new material treated in the same manner.

As the specimens originating from the different strata varied exceedingly in habit and, in consequence of this, in surface, in order to compare the results the numerical data obtained regarding the animals found in the coating were calculated to one given surface (6.3 dm^2 = the surface of 10 pieces of 20 cm long reed stem). The surface of the plant was determined in the following way. From examples collected from the different strata and different localities, I classified the leaves and stems according to dimensions into different types (Both of *Myriophyllum* and *Potamogeton* 3 leaf, 2 stem types). From each type 10—10 examples were measured as to surface. After this I calculated the average surface characteristic of the type. The surface of the samples whose coatings were investigated quantitatively were determined with the help of the average type surfaces described, and data on the sample itself (type, leaf-number, stem-length). After this the numerical data regarding the animals found in the coating were reduced to the average surface (6.3 dm^2) (See Table II). It may be noted that the average surface of an entire plant was also estimated: *Potamogeton* in the Kis-öböl has $\pm$ 15 dm^2, in the open water $\pm$ 15 dm^2; *Myriophyllum* in the Kis-öböl has $\pm$ 4 dm^2, in the open water $\pm$ 26 dm^2.

A statistical elaboration of the results of my investigations was provisonally not carried out, because the nature of the collections was not appropriate for this purpose, and because the number of collections made in the same circumstances was small.

Let us now examine the coating more closely. (See page 17.)

I classified the coatings on the basis of my observations according to thickness, in 5 empirical groups: Where the coating is very

thin: 1x; somewhat thicker, sufficient to be easily remarked, 2x; fairly well developed, in the axil of the leaves a mass of sediment, 3x; thick coating, here and there a little woolly, 4x; the richest woolly coating which covers both stem and leaves, 5x.

We can scarcely speak of a coating on *Potamogeton* in the "a" stratum. Here at most the leaves are covered with a thin calcareous crust which is caused by assimilation (1x). Lower we find constantly thicker coatings (2x—5x), consisting principally of mud, detritus and Diatoms; this at 2—3 cm from the base of the plant is again thinner. The coating on *Myriophyllum* is never as rich as on *Potamogeton* but it seems more equally distributed (2x—4x). In this case a thin coating is also found near the surface (2x) of the water, the calcareous crust being entirely lacking. Lower the coating on *Myriophyllum* too is thicker (3x—4x) but near the bottom there are some stems without leaves entirely free of coating. Immediately above the bottom some *Cladophora* are to be found. In other cases a hard crust with a pitted surface is found (1x) which consists principally of cocoons of *Herpobdella,* small *Dreissena* and oval-shaped, very strongly attached Diatoms, which last often cover everything evenly.

The difference in coating formation between *Myriophyllum* and *Potamogeton* may partly be explained by the different habit of the plants, but partly may be due to the precipitated calcareous particles on *Potamogeton.* The roughness thus caused makes *Potamogeton* better suited for forming a coating, besides which it collects much sediment at the axil of its leaves. Because of the stiffness of the stem of *Potamogeton* and the undivided leaves, the waves more successfully attack this plant near the surface of the water and there can more easily wash away the coatings. On *Myriophyllum* a calcareous formation cannot be observed, and so its surface is perhaps less well adapted to forming coatings. Again, the stem of the plant is very flexible and its divided leaves "give" to the movement of the waves. Thus the coating can remain on them even near the surface of the water.

So far the quantity of the mass coating has been established only by careful observation and estimation; exact quantitative measurements are still lacking.

As will appear from Figure 3, the quantity and quality of the coatings seem to be in close relation to the number of inhabitants. This is true especially in the case of organisms with the least locomotion. These (Nematoda, Oligochaeta, Chironomida larvae above ± 2 mm, Chironomida pupae, adult *Corophium* and *Dreissena)* are always the

most frequent near the bottom. If we take the occurrence expressed in percentage (A), in the open water these comprise 82.5%, of the organisms on *Potamogeton,* 90.3% of those on *Myriophyllum;* therefore on both an average of 86.9%. This can be explained by the fact that in the "c" stratum of the deep water the movement of the water is the least effective and thus the coating can most richly develop (4x, 5x). In the Kis-öböl the bottom water is more often disturbed because of

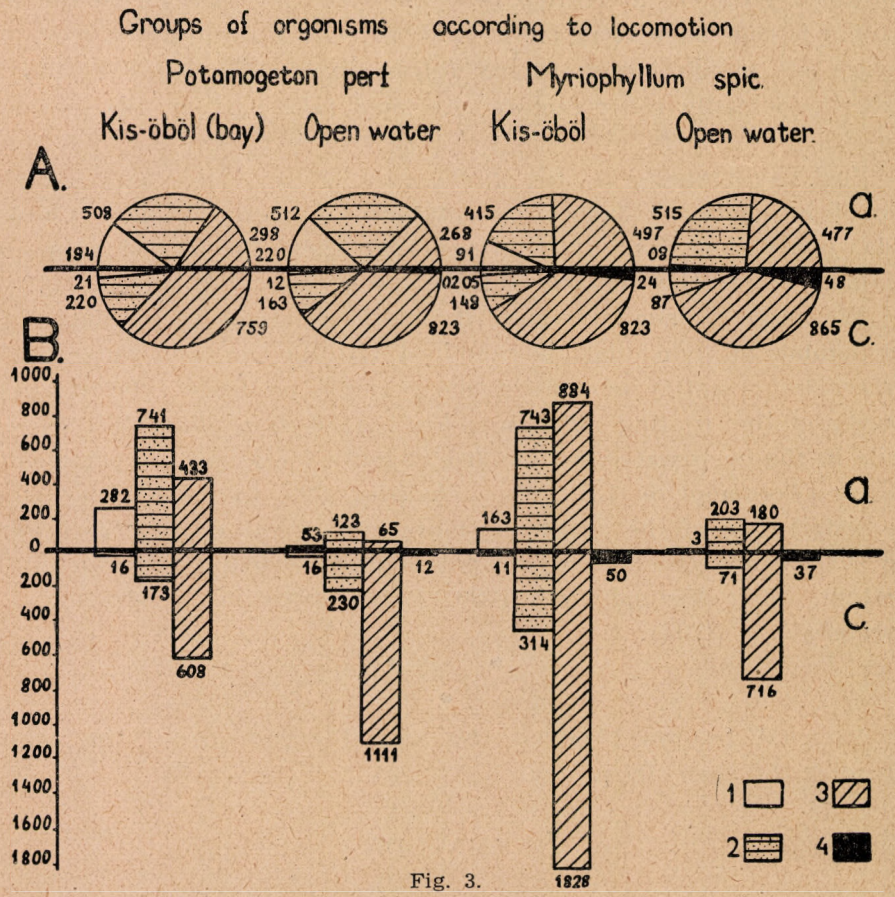

Fig. 3.

Groupings of organisms according to locomotion. Occurrence of the various types on different substrata and localities in % (A), number of individuals (B). 1.) Most active organisms (Sida, Acroperus, Alona) 2.) Active organisms, creeping and swimming on the surface of coating. (Turbellaria, Rotatoria, Cyclops, juv. Chironomidae, juv. Dreissena, juv. Corophium, Ephemerida larvae.) 3.) Less active organisms, living in the coatings and feeding on it (Chironomida larvae) $<$ 2 mm, (Polycentropidae, Nitocra, Punctodora, Oligochaeta) 4.) Sessile organisms (Adult Dreissena, Helobdella cocoons).

the bay's situation and shallowness. Here this group of organisms is not as frequent as in the open water (*Myriophyllum* 82.3, *Potamogeton* 75.9, average 79.1%). (Thickness of coating, 2x, 3x.)

Considering the same group of animals having the least locomotion much scantier results are obtained near the surface of the water. As has been mentioned, the coating on *Potamogeton* is the sparsest in the open water near the surface (1x). The percentage of the above-mentioned group is in reality here the smallest (26.8%), in the protected Kis-öböl somewhat more (29.8%). On *Myriophyllum* near the surface we obtain larger values (open water 47.7, Kis-öböl 49.7%). (2x). Undoubtedly other factors also may take part in this phenomenon.

Just the contrary is true in the case of organisms with a higher rate of locomotion (Cladocera, Rotatoria, Turbellaria, juv. Chironomida, juv. *Corophium,* juv. *Dreissena).* In these the largest values are obtained nearer the surface and the lowest near the bottom.

We arrive at similar results if not the relations in percentage but the number of individuals is examined (B). In the open water on the surface the quantity of individuals is small, in direct relation to the paucity of the coating (*Potamogeton* 231, *Myriophyllum* 385. 1x, 2x), while in the deep water the number of individuals is disproportionately greater (*Potamogeton* 1372, *Myriophyllum* 822). In the Kis-öböl where the distribution of the coating is more even, great differences between the surface and the bottom are not to be expected, (*Potamogeton* "a" stratum, 1496; *Myriophyllum* 1790; *Potamogeton* in "c" stratum, 796; *Myriophyllum,* 2202).

* *
*

In what follows I review the animal organisms of the coating according to their frequency.

NEMATODA

In all the coatings of *Potamogeton* and *Myriophyllum* great numbers of Nematoda were to be found at each collecting place. These comprise 34% of the entire material. Of 10,542 Nematoda, 33 belong to the large, *Dorylaimus* genus, all the rest are *Punctodora* size, most of them *Punctodora* sp.*

* Elsewhere when *Punctodora* is mentioned, *Punctodora* -sized Nematoda are to be understood.

The relation which can be established between the quantity of the coating and the organisms living in it is specially well demonstrated by the *Punctodora*. It can be seen in their distribution in *Myriophyllum* and *Potamogeton* coatings:

25

	Punctodora			Metazoa including Punctodora			
	Iindivid²	%	Ind/dm²	Individ	%	Ind/dm²	Thickness of coating
Myriophyllum	3431	32.6	50.0	10994	34.6	145.3	2x
Potamogeton	7078	67.4	79.7	20850	65.4	231.0	3x

As will be seen later, it is apparent that the minimum of *Punctodora* in stratum "a" and maximum in stratum "c" depends also on the quantity of the coating.

HARPACTICIDA

After the *Punctodora* the most frequent are the Harpacticida. In the entire material there were 3698 individuals (11.6%, per dm² 22.6 individuals). The *Nitocra inuber* were overwhelmingly greater in numbers than any other species. Of *Ectinosoma Edwardsii* only 8 specimens were found, and of *Canthocamptus staphylinus* only a couple. In every collection *Nitocra* was to be found on *Potamogeton* as well as on *Myriophyllum*. It appears that on *Myriophyllum* fewer individuals live than on *Potamogeton (Myriophyllum* 21; *Potamogeton* 26.5 per dm²). It was definitely to be observed that in the Kis-öböl they were much more frequent (altogether 2416, 35/dm²) and in the open water more rare (1282, 13.5/dm²). In the Kis-öböl they were the most frequent in stratum "b"; in the open water their number increased gradually with the depth, reaching the maximum in the "c" stratum near the bottom. It is probable that the distribution of the *Nitocra* specimens — regardless of the locality, depth and plant — depends as in the *Punctodora* on the quantity of detritus in the coating, its chief source of nourishment. *Nitocra* nauplius, as well as other young organisms, (juv. *Corophium*, sphaerium form of *Dreissena)* were to be found only in the Kis-öböl and in the upper strata of the open water. The nauplius of *Nitocra* penetrates into the interior of the coating and there carries on its peculiar shaking movements. Normally this movement of the inorganic particles and detritus in the coating calls attention to the tiny, hardly

perceptible creatures. But an exact count of them in the specially thick coating is very difficult even when they are alive, therefore their numerical data is not an exact valuation. The metanauplius can be distinguished from the adults by the large red spots which are already present in the eggs and the nauplius. The metanauplii and the adults swim swiftly among the particles of the coating and in the cavities. In the Petri dish the greater mass of them assembles in the darkest part, while a smaller number swims towards the light. The females with eggs also without exception sought out the darkest places.

COROPHIUM and DREISSENA

A really great number of specimens of one of the most common Amphipoda of the Balaton, *Corophium curvispinum* G. O. Sars forma devium Wundsch (3232, 10.2%, 19.7 ind/dm²) was present, as well as *Dreissena polymorpha* Pall, the zebra mussel (2289, 7.2%, 14.0 ind/dm²). Between these two organisms there is a very close relation. Both are of pontus origin, and both increased to an enormous extent in the Balaton during the 1930s. (Entz-Sebestyén, 1946. p. 301—302). In 1931 when A. Meschkat investigated the reed-coatings of the Balaton he did not find a single example of any of them, while since then they are to be found very frequently (on reed too). On the plants now studied these two species were everywhere found. In one case only in the whole collection both were lacking, from *Myriophyllum* in the "a" stratum in the Kis-öböl. In one other case also, from *Myriophyllum* from the same place only *Corophium* was to be found, and of that only 6 examples. Adult examples of both species attach themselves fast to their substratum. The *Dreissena* fastens itself to the stem and leaves of the plants with byssus thread, while the *Corophium* builds its tube in the same places. Where the *Dreissena* occur in quantities they settle on one another, making clumps on the stems, usually just above the bottom. The *Corophium* prefers the angles of shells in building its tube. They seem to be alike as to their food and some life conditions. On the *Myriophyllum* both organisms occur in smaller numbers. But while the *Dreissena* in the adult stage seldom moves about, the young as well as the adult *Corophium* are good swimmers. In the "a" stratum neither of them occurs in quantity (Table II.). The examples to be found in stratum "a" are very young *Corophium* and the sphaeriums of *Dreissena;* adult types of both are found in "b" stratum, but still more in stratum "c".

In the *Dreissena* we see a gradual increase in numbers in the lower parts of the stem ("a" stratum 1.1, "b" stratum 8.1, "c" stratum 34.0 ind/dm²), both in the bay and in the open water (see below). But in the Kis-öböl there were fewer specimens than in the open water (Kis-öböl 5.9, open water 19.6 ind/dm²).

We find slightly different conditions among the *Corophium*. Here too can be observed in the open water the gradual increase of examples according to depth ("a" stratum 3.2, "b" 9.6, "c" 41.7 ind/dm²), but in the bay the data strongly differs from this, for it was in the "b" stratum that the greatest number of specimens was found. This can perhaps be explained by the fact that in the Kis-öböl the number of young specimens was greater (ENTZ B. 1943 p. 37). In open water, where proportionately more adult than young *Corophium* are found (open water, adults 15%, Kis-öböl 6.2%), the increase in numbers of *Corophium* in the lower depths is more clearly to be seen.

Numbers of individuals of *Dreissena, Corophium* and *Nitocra* estimated to a surface of 1 dm² of the substratum: Open water 1) *Myriophyllum,* 2) *Potamogeton;* Kis-öböl, 3) *Myriophyllum,* 4) *Potamogeton.*

	Dreissena				Corophium				Nitocra			
Stratum	a	b	c	average	a	b	c	av.	a	b	c	av
1.	0.0	0.8	14.0	4.9	0.0	3.3	5.1	2.8	38.1	56.2	18.4	37.6
2.	0.8	2.2	4.8	2.6	4.1	69.2	19.7	31.0	25.9	45.9	39.9	37.2
3.	0.3	6.2	18.4	8.2	4.0	0.3	61.6	25.3	2.7	12.9	14.0	9.9
4.	0.0	7.8	52.5	20.1	1.0	9.0	21.8	10.6	0.15	2.0	9.9	4.0

If we take only the adult *Corophium* into consideration, the distribution is as follows:

Stratum:	a	b	c	average
Kis-öböl	0.0	2.2	0.8	1.0
Open water	0.08	1.3	6.4	2.6

CHIRONOMIDA

Chironomida larvae are also constant inhabitants of the coatings. Among them we find red ones in smaller numbers and colourless in masses (see ENTZ-SEBESTYÉN, 1946, p. 348). The red ones are to be found round the middle of September. Thus on the 13th of September 65 specimens were found, later only one. They occurred prin-

cipally on Potamogeton, always near the bottom (*Potamogeton* 48, *Myriophyllum* 18 ind.).

Of colourless specimens altogether 2756 were found, of which 1/5th (542) were ± 2 mm long or longer. The rest were very small, very active, and as transparent as water. Pupae occurred principally near the surface of the water (34 ind.). The young larvae moved about quickly in the Petri dish, and most of them assembled on the light side. In general in the daytime collections the most Chironomida would be found in stratum "b" (24.3 ind/dm²). Fewer examples would be found in "a" stratum (11.1 ind/dm²). In the evening collections in the "c" stratum many young specimens were found (504), and at this time many less in the upper strata. This can be explained by the young larvae's sensibility to light and their ease of locomotion. They occur with equal frequency on both substrata (*Potamogeton* 15.7 ind/dm²; *Myriophyllum* 15.5). But they seem to be more frequent in the bay (20.6 ind/dm²) than in the open water (9.16 ind/dm²).

OLIGOCHAETA

A well distributed group is formed by the Oligochaeta, whose members creep about, swim in and feed off the coating in large numbers. Their total number is 3309, 10.5% of the total material. Several species of several genera are to be found. Their distribution varies. The most common are the species of the genus *Nais*. It is characteristic of them that they live everywhere in the coating, both in the Kis-öböl and in the open water, but never in large numbers. In their distribution as to depth they seem to resemble the *Corophium*. They avoid stratum "a"; in the bay they frequent the "b" stratum, in the open water the "c" stratum (Kis-öböl, "b" stratum, 94.8 ind/dm², "c" 35.2 ind/dm²; open water, "b" 42.3; "c" 87.5 ind/dm³). In the Petri dish they seek the light. Altogether I noted 1508; on an average of 8.7 examples per dm² surface.

Stylaria lacustris occurred almost in the same number — 1146 examples; 6.7 per dm². Most of them were collected in the bay (966, 13.9 ind/dm²) and only a small number (133, 1.4 ind/dm²) in the open water. The *Stylaria* also avoids stratum "a" and likewise is common in "b" and "c" strata.

Decidedly more rare is the *Chaetogaster langi*. The greater number occurred on *Potamogeton* (573, 1.9 ind/dm²) and only a very few on *Myriophyllum* (77, 1.1 ind/dm²). This fairly small *Chaetogaster* feeds

exclusively on the Diatoms living in the coating. Much larger than this is the *Ch. diaphanus*, of which 10 examples were found on *Potamogeton*. The Oligochaeta mentioned penetrate with their strong muscles into the interior of the coating and a gentle rinsing does not remove them. The fairly frequent *Aeolosoma quaternarium* has not such strong muscles. As is well known, it moves principally not with muscles but with cilia (WESENBERG-LUND, 1939, p. 317). Though this also penetrates with very slow movements into the interior of the coatings, rinsing will wash away most of them. It occurs in the bay and in the open water, both on *Myriophyllum* and *Potamogeton*. In the "a" stratum with the exception of 1 case it was not to be found (Kis-öböl, *Myriophyllum*, VIII 30) and in the "b" stratum it was also rare. It was the most abundant at the bottom.

ROTATORIA

All the members of the groups so far discussed stand in close relation to the water plant, or rather to its coating, being at the same time very essential elements of the latter. They live in the coating, build their homes in it, feed on Diatoms living there, or on detritus deposited in it, or attach themselves immediately to the water plant as substratum. The representatives of another group, the Rotatoria (2681, 8.5%, 20.9 ind/dm^2), swim and creep on the surface of the coatings, penetrating into its cavities. Rinsing removes them easily from the coatings. More than two-thirds of the Rotatoria belong to the genus *Notommata* (EYFERTH-SCHOENICHEN II. p. 377—79), the others (790) are of various genera. These latter do not form a united ecological group and thus are not suitable for comparison. It is easy to recognize the *Notommata* which feed on Diatoms by their larger size and their relatively slow movements. Investigating their occurrence from the standpoint of substratum, time and place of collection, and depth, certain relationships may be recognised. For these comparisons I used only the data from collections I, II, IV and V, as later their numbers diminished, at first suddenly and later more gradually. In examining their appearance in the different strata, whether collectively, whether according to plant species *(Myriophyllum, Potamogeton)*, or whether to locality (Kis-öböl, open water), we can see that the numbers diminished in relation to depth (stratum "a" 41.3 ind/dm^2; "b" 19.7 ind/dm^2, "c" 8.2 ind/dm^2. (Fig. 4 A.) But the same regularity is apparent, not only in the numbers of individuals of *Notommata* found but

also in the percentual proportion to the other organisms. However the diminution according to depth is not evident in the case of evening collections: (Fig. 4 B.). On the evening of October 1st the differentiation according to strata could not be observed.

According to locality it seems that the *Notommata* are more characteristic of the bay (33.5 ind/dm²) than of the open water (7.7 ind/dm²). Some differences can also be distiguished according to substratum (*Myriophyllum* 24.5 ind/dm², *Potamogeton* 16.8).

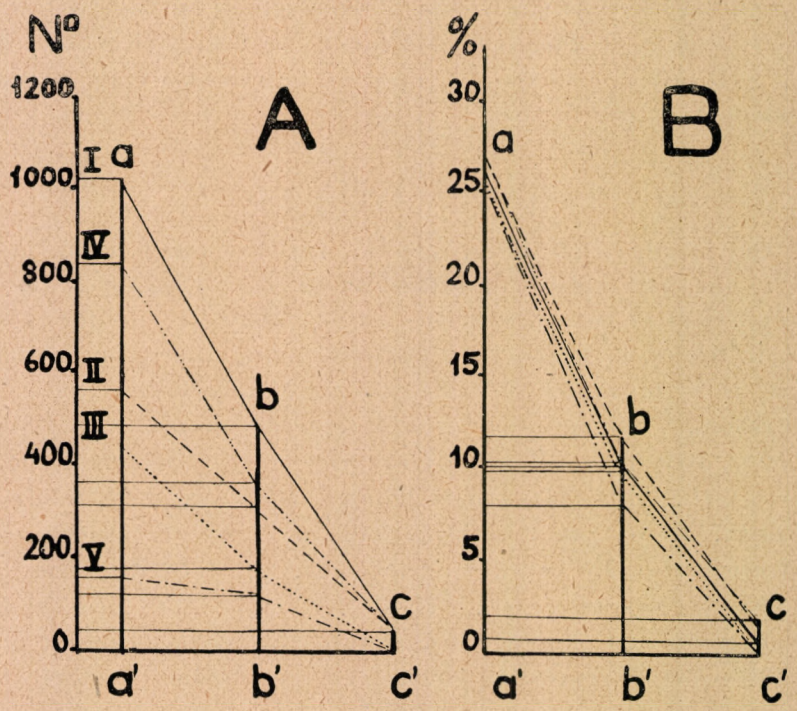

Fig. 4.

Occurrence of Notommata (B in % and A number of individuals), according to depth (a—á';, b—b';, and c—c'; strata), locality and substratum, from the four first collections. I (————) total number, II (— — — —) on Myriophyllum, III. (.....) on Potamogeton, IV. (..—..—) bay, V. (.—.—.—) open water.

POLYCENTROPIDA

The occurrence of the Polycentropida, which belong predominantly to the species *Ecnomus tenellus,* (Rousseau E. 1921, p. 513.) forms — so to say — a mirror-picture of that of the *Notommata.* By this is

meant that where *Notommata* are the most numerous Polycentropida are entirely lacking, and vice versa.

It is possible that it was only by chance, but it is interesting to note that where the Polycentropida formed more than 3% of all animals, the *Punctodora,* in other places present in large masses, were almost entirely lacking (scarcely 7% of all animals). However I could establish no direct relation between these organisms.

The Polycentropidae are carnivorous. In the Petri dish they sometimes ate up Chironomida larvae. Under the microscope they always fled into obscurity, and most of them assembled at the point the furthest distant from the light. They seem to prefer the deepest and darkest regions of the Balaton; they are almost entirely lacking in the Kis-öböl (1.7 ind/dm²), while in the open water they are much more frequent (5.8 ind/dm²). In the open water they are the most common in the "c" stratum (11.3 ind/dm²), in strata "a" and "b" they are rare ("a" stratum only 1—2 examples; "b" 2.7 ind/dm²). They occur much more frequently on *Potamogeton* than on *Myriophyllum (Potamogeton* 6.9 ind/dm² *Myriophyllum* 1.7). The most and biggest Polycentropida larvae were to be found in the muddy sediment deposited in the leaf axil of the *Potamogeton,* in which the specimens were almost entirely hidden.

At the evening collections they were present in larger numbers in the upper strata too. Therefore it is possible that these active and seemingly negative phototropic organisms, like the *Notommata* and the Chironomida, come to the surface of the water in the evenings.

The numerical distribution of Polycentropida on October 1st, 1946, per dm²:

	Morning	Noon	Evening
"b" st.	7	15	73
"c" st.	108	119	217

CLADOCERA

Two species of Cladocera play a great role in the coatings, *Sida crystallina* (990 individuals, 3.1%, 6 ind/dm²) and the *Acroperus harpae* (199, 0.6%, 1.2 ind/dm²). Both water fleas live in the upper strata of the water. *Acroperus* is frequent on *Myriophyllum* (2.2 ind/dm²), hardly occurring on *Potamogeton* (0.5 ind/dm²), while *Sida* is almost exclusively found on *Potamogeton* (11.0 ind/dm²). On

Myriophyllum it is very scarce (0.3 ind/dm²). The swimming of *Acroperus* is slow; it is rather a creeping on the coatings. In both localities it is the most common in stratum "a"; in "b" and "c" only one or two specimens are found. Regardless of locality the mean values of occurrence of *Acroperus* in the different strata are: "a" 6.6, "b" 0.4, "c" 0.08. They occurred principally in the Kis-öböl. The largest number of examples was noted there on *Myriophyllum* in stratum "a" (23 ind/dm²). In the open water it is much more rare (0.2 ind/dm²). The *Sida,* which adheres to the surface of *Potamogeton* and is a very good swimmer, is principally an open-water organism (open water 7.8 ind/dm²). On *Potamogeton* growing in both localities it was frequent in the two upper strata ("a" 12.2, "b" 11.4 ind/dm²), while in "c" stratum there were only a few examples (0.1 ind/dm²). However there are some differences as to locality; in the bay the largest masses were in the "a" stratum, while in the open water they were to be found in "b" stratum. The same phenomenon, that the maximum numbers of individuals occur in different depths in the bay and in the open water, is to be observed in other organisms too (see p. 26, 28).

TURBELLARIA

Under the microscope more of the smaller Turbellaria occurred fairly frequently (307, 1.0%, 1.9 ind/dm²). Though their number is never great they are to be found everywhere; the greater part of them belong to the genus *Dalyellia,* though other species were also observed. All of them are small worms which feed on Diatoms and swim above the coatings or in their cavities. In the Kis-öböl more (3.7 ind/dm²) and in the open water fewer (0.6 ind/dm²) were found, in equal numbers on *Myriophyllum* and *Potamogeton.*

CYCLOPS

As to frequency, *Ciclops strenuus* follows in rank, more than 100 specimens of it being found in the total material investigated. This swims here and there, "grazing" on the surface of the coatings, and swims away on the slightest disturbance. They occurred in about equal numbers in all collections.

There were also numbers of other organisms in the material collected but always so few in number that no regularity in distribution could be established. Hydra *(Pelmatohydra oligactis?) Alona sp.* were

seen here and there. Ephemerida larvae (44 examples) occurred only in the Kis-öböl and there too an Ostracoda. With the plants growing in the open water were gathered some members of the plankton such as *Diaptomus gracilis, Leptodora Kindtii, Daphnia cucullata,* etc.

*

* *

In working up the material it was apparent that with the first rinsing (see method) it was always the same organisms which were washed away in great numbers. In order to investigate this more closely, the samples from 12 collections were gently rinsed, later subjec-

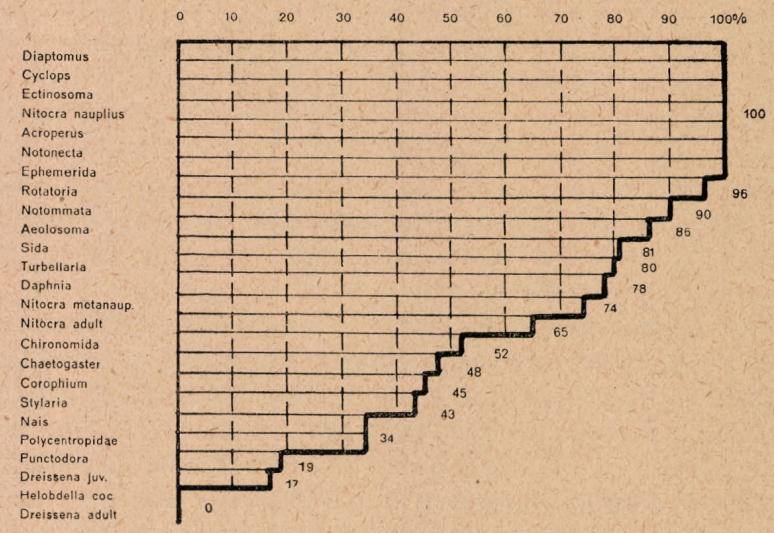

Fig. 5.

Percentual distribution of the animal organisms exhibiting degree of adherence when rinsing the plant. (For numbers see text.)

ted to strenuous shaking and rubbing. The number of organisms gained by this were compared. (Fig. 5.) The result was that the most active organisms were found to be those which could be 100% washed away by the first rinsing, meaning that they were not so closely related to the coating *(Cyclops, Acroperus,* juv. *Notonecta,* etc.).

80—90% washed away were those which, also active organisms, rest from time to time on the plant or on the surface of the coating *(Sida, Notommata,* etc.). These groups originated mostly from strata

**

"a" and "b", being their characteristic inhabitants. Least easily washed away were the organisms living in the interior of the coating. These are characteristic of stratum "c" *(Punctodora,* Oligochaeta, *Dreissena,* etc.). As we see, this succession throws light on the degree of their locomotion, their methods and degree of adhering (from time to time, or permanently). But it appears also that in the upper strata occur principally the very active organisms. Near the bottom, on the other hand, occur those types which are in close relation to the water plant or its coatings.

The feeding conditions of the animals of the coatings are very interesting. The majority of them feed on detritus (79.8%). These are primarily the tiny sessile Nematodes, the sessile *Dreissena,* the tube-making *Corophium,* besides these the *Nais, Stylaria, Aeolosoma, Nitocra,* etc., which creep in the interior of the coating, and probably the greater part of the Chironomides. 11.9% of the animals feed on Diatoms. The greater part of these are animals easy of locomotion, swimming or creeping on the surface of the coating or in its cavities, such as the Rotatoria, *(Notommata)* Turbellaria, principally members of the genus *Dalyellia,* the *Chaetogaster langi* and the commonest *Cyclops, Cyclops strenuus.* Half of the remaining (4%) are carnivorous, primarily the Polycentropidae, which, at least in captivity, eat the Chironomidae larvae. The other half (3.8%) consist of Cladocera, primarily *Sida crystallina* and the *Acroperus harpae,* of which it is known that they are "minute feeders", or microphagous animals (CARPENTER 1928 p. 58). It is to be remarked that in the entire material there were found only 5 examples of Lepidoptera larvae, (LAMPERT K. 1904 p. 87), which feed on the leaves of the plants. Thus the great majority of the animal organisms of the coatings employ the water plant only as a substratum, while they find their nourishment in the coating itself.

Let us assemble the resemblances and the differences from one another between the coatings of 1) *Myriophyllum* and *Potamogeton,* 2) of the Kis-öböl and the open water, and 3) of the "a", "b", and "c" strata.

1.) We see from Table III that the coatings on *Potamogeton* harbour more animals than do those of *Myriophyllum.* This very likely is connected with the development of the coating as such, which is richer on the *Potamogeton.* Thus, *Punctodora, Nitocra, Corophium, Dreissena* and the *Nais* (that is, the organisms most closely attached to the coatings) are naturally more frequent on *Potamogeton,* though

Stylaria and Chironomida larvae (which would seem to be also in close relation to the quantity of the coating) live in equal numbers on *Myriophyllum* and *Potamogeton.*

All these organisms — occurring on both plants — are such as are most closely attached to the coating. On the other hand, *Sida* (*Potamogeton* 11.0 ind/dm², *Myriophyllum* 0.3) and the Polycentropida (*Potamogeton* 11.6, *Myriophyllum* 1.3 ind/dm²) live almost exclusively on *Potamogeton,* while *Acroperus* (*Potamogeton* 0.5, *Myriophyllum*

TABLE III.

Species or group	stratum „a"	stratum „b"	stratum „c"	Potamoget	Myriophyllum	Kisöböl	Open water	Average
1. Punctodora sp.	34.5	31.5	56.0	79.7	50.0	78.6	56.8	64.2
2. Nitocra inuber.	16.7	34.4	24.2	26.5	21.0	37.9	12.3	22.6
3. Corophium curv.	3.2	18.0	29.0	22.9	15.4	18.3	21.6	19.7
4. Chironomidae	11.1	24.3	2.8	15.7	15.5	21.0	9.4	17.4
5. Notommata sp.	40.3	19.7	8.2	16.8	24.5	33.5	7.7	16.4
6. Dreissena polym.	1.1	8.1	34.0	15.7	10.9	5.9	19.6	14.0
7. Nais sp.	3.1	11.1	6.5	11.1	4.5	8.6	7.9	8.7
8. Polycentropidae	0.2	2.7	11.3	11.6	1.3	2.5	10.5	7.2
9. Stylaria lacust.	2.3	15.4	16.1	6.1	7.8	14.2	1.1	6.7
10. Sida crystallina	12.2	11.4	0.1	11.0	0.3	4.3	7.8	6.0
11. Rotatoria	14.0?	5.7?	5.9?	4.8?	6.3?	9.0?	2.1?	4.8?
12. Chaetogaster lan.	1.2	2.4	3.1	5.3	1.1	4.1	2.8	3.5
13. Turbellaria	4.3	1.2	4.5	1.9	2.1	3.7	0.6	1.9
14. Aeolosoma quater.	1.5?	0.9	1.6	1.1	1.4	1.6	1.0	1.2
15. Acroperus harpae	6.6	0.4	0.0	0.5	2.2	2.5	0.2	1.2
16. Cyclops sp.	0.8	1.6	0.1	1.2	1.0	1.0	1.2	1.1
Total	153.1	188.8	203.4	231.9	165.3	246.7	162.6	196.6

Average number of individuals of the most frequent species or groups according to strata, substrata and locality calculated to 1 dm² plant surface. For calculating the math. mean data the whole material of all the collections was used, except the mean regarding the depth, in calculating which only Collections I, II, V were considered.

2.2 ind/dm²) prefers *Myriophyllum.* Both organisms are less closely attached to the coating. Probably there is some hidden relation between these organisms and their substratum but of what nature I was unable to determine. The Turbellaria, *Aeolosoma* and *Cyclops,* being organisms but loosely attached to the coating, are equally frequent on both substrata.

2.) There is scarcely any difference between animal members of the coating — in a qualitative sense — in the open water and the Kis-öböl, but quantitative differences can be remarked in the case of some organisms. Thus the greater number of *Nitocra inuber, Notommata,* and nearly all *Stylaria.* Turbellaria and *Acroperus* derive from samples of the Kis-öböl, while the greater number of *Dreissena* and *Sida* and nearly all Polycentropidae were to be found in the open water. In somewhat greater numbers were *Chaetogaster* and Chironomida larvae and the *Punctodora* in the bay; the *Corophium* in the open water.

3.) Where strata are concerned, several different types of differentiation can be made. Some organisms have their maximum in stratum "a" everywhere, and their numbers diminish precipitously in the lower strata. Such are *Notommata* and *Acroperus.* Others frequent preferably the lower depths of the open water, as of the Kis-öböl. This is specially characteristic of the *Sida* (Kis-öböl the maximum in "a" stratum; open-water maximum in "b" stratum) but the same can be observed of *Nitocra, Nais* and *Corophium* and Chironomida larvae. These last occur in the bay, all in stratum "b", while in the open water they are the most frequent in stratum "c". The cause of the difference can be sought in wave action.

Another group consists of those organisms whose numbers increase according to depth, in both collecting places. These always dwell in "c" stratum (Polycentropidae, *Dreissena).* Some organisms are also known which are distributed equally in all strata.

SUMMARY.

The coatings on small growths of *Potamogeton* and *Myriophyllum* in the open water and a bay of Lake Balaton, and the animal organisms living in them, were examined on the basis of material taken from different strata during 5 weeks at the beginning of the autumn of 1946. The organisms were examined and numbered in a live condition, and the numerical data uniformly calculated to a given surface. On the *Potamogeton* the coatings are very thin near the surface and very rich near the bottom. The difference is more remarkable in the open water than in the bay. On *Myriophyllum* the coating is not so rich but more equally distributed. Characteristic organisms living in the coating according to frequency are: *Punctodora, Nitocra* sp. *Corophium curvispinum, Chironomida* larvae, *Notommata* sp., *Dreissena poly-*

morpha, etc. The differences between the animals harboured by the coating of *Potamogeton* and *Myriophyllum* are chiefly quantitative, although some qualitative differences were also observed. The organisms in closest relation to the coating live principally near the bottom, those more independent of it in the upper regions. A relation can be shown between the rate of locomotion of the organism and the ease with which it is washed away. The majority of the inhabitants of the coating feed on the detritus in the coating, or on Diatoms or other animal organisms living in it, and only one or two specimens of a very few species were found to feed on the plant itself.

I take this occasion to express my deep gratitude to Dr. Olga Sebestyén, Private Docent of the University of Budapest, for her constant help and advice throughout the course of this work, and to Mrs. J. Thompson Vass for the English translation.

REFERENCES.

1. Boldt W. (1940). *Oligochaeta in* Schultze: *Biologie der Tiere Deutschlands.* **12**a 1—39.
2. Brauer A. (1909—11). *Die Süsswasserfauna Deutschlands.* Jena.
3. Carpenter K. E. (1928). *Life in inland waters.* p. 58.
4. Dahl F. (1925—42). *Die Tierwelt Deutschlands und der angrenzenden Meeresteile.* Teil 1—40.
5. Entz B. (1943). *Magy. Biol. Kut. Munk.* **15.** 37.
6. Entz G.—Sebestyén O. (1946). *Magy. Biol. Kut. Munk.* **16/2** 179—411.
7. Lyferth—Schönichen (1927). *Einfachste Lebensformen des Tier und Pflanzenreichs.* Berlin **1—2.** 377—79.
7a. Krecker, F. H. (1939). Ecology 20 p. 553—562.
8. Lampert K. (1904). *Az édesvizek élete.* 87.
9. Lenz F. (1928). *Biologie der Süsswasserseen. Berlin.* 115.
10. Lucks R. Rotatoria in Schultze: *Biol. d. T. D.* **10.** 1—176.
11. Meschkat, A. (1934). *Arch. f. Hydrobiol.* **27.** 436—521.
— — (1934). Magy. Biol. Kut. Munk. **7.** 154—63.
12. Reisinger, E. *Turbellaria in* Schultze: *Biol. d. T. D.* **4.** 1—64.
13. Rousseau, E. (1921). *Les larves et nymphes aquatiques des insectes d'Europe.* 513.
14. Ruttner, F. (1940). *Grundriss der Limnologie. Berlin.* 118.
15. Spandl, H. Copepoda in Schultze: *Biol. d. T. D.* **15.** 1—78.
16. Storch, O. Cladocera in Schultze: *Biol. d. T. D.* **14.** 23—102.
17. Ulmer, G. Trichoptera in Schultze: *Biol. d. T. D.* **36.** 1—113.
18. Wesenberg—Lund, (1939). *Biologie der Süsswassertiere Wien.* 317.
19. Wülker G. Nematodes in Schultze: *Biol. d. T. D.* **8.** 1—64.

THE TARDIGRADA FAUNA OF THE TIHANY PENINSULA.

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With 1 Figure and 2 Tables in the text.

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At the request of the late Professor G. Entz, investigations were made as to the water bear fauna of Lake Balaton and surroundings. In the beginning the collections were but occasional. However in June and July, 1943, I had the opportunity of carrying out thorough studies. As a guest of the Hungarian Biological Research Institute at Tihany I made regular collections on the Tihany peninsula (Fig. 1.) and visited the water bodies in the environment. The laboratory work was done at the same Institute. Beside collecting faunistical data attention was given to ecological conditions and to the biology of water bears.

Tardigrada, according to the literature, as well as in my own judgment, might be arranged in the following ecological groups:

1. Xerophil forms, which endure extreme conditions of certain factors, living in moss cushions and *Sedum* "lawn" in very sunny places which are frequently subject to continued dryness.

2. Sciophil forms living in shady and humid habitats, where lasting dryness never occurs.

3. Hygrophil forms from irrigated localities, where water supply is steadily secured and permanent dryness never occurs.

4. Hydrophil forms living in water, among weeds or in detritus well soaked with water.

5. Eurytop forms, capable of adapting themselves to various milieus, at home in water as well as in dry localities.

The habitats of water bears in the peninsula might be grouped according to the chief minimum factor necessary to these animals, which is the degree of moisture.

I. The most divers Tardigrada fauna may be found in localities having extreme conditions in that respect, such as moss cushions grow-

ing on the gravel earth of barren hillsides, on rocky hilltops, on roofs of houses, on meadows and on dry stone walls, all of them well exposed to the sun. Predominant species are *Macrobiotus montanus, M. Hufelandi, Echiniscus canadensis, E. testudo, Hypsibius Oberhäuseri,*

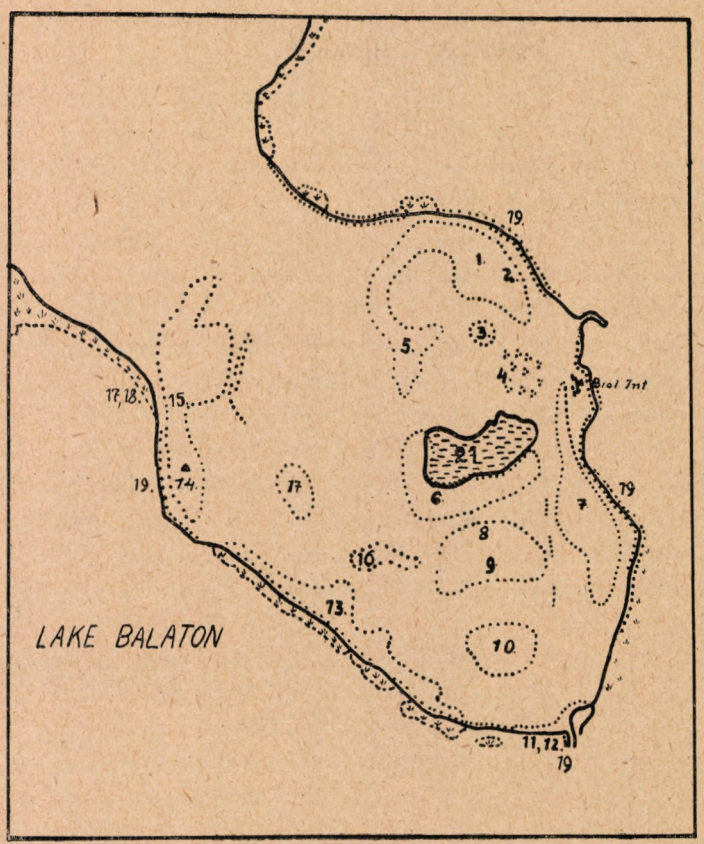


Fig. 1. Map of Tihany peninsula showing localities investigated for Tardigrada. For meaning of numbers see Table II. Border of habitats.....

Milnesium tardigradum. Echiniscus bisetosus, Macrobiotus forciger, Hypsibius brevipes were found only in such habitats. Most of the species are xerophil ones.

II. In thick moss cushions of shady humid habitats, such as tree trunks and soil in woods, fewer species could be enumerated though here conditions of existence would seem to be more favourable. Only one species *(Hypsibius Dujardini)* was found here which did not occur in habitat I. *Pseudechiniscus* species are wanting.

III. In the damp surroundings of the Cyprian spring, located about 100 m distance from the lake shore, only two species could be recorded: *Macrobiotus Richtersii* and another species of the same genus, represented by one single specimen which was not in condition appropiate for identification.

A very interesting and rich fauna is harboured by the lake shores which are protected by a stone wall. The moss cushions scattered here may be arranged in three zones. The upper one is irrigated only in the worst storms, most of the time is subject to dryness. The middle one is frequently irrigated by the waves, but when the wind is still it dries thoroughly. Moss cushions growing here are thick and clear and harbour a rich Tardigrada fauna. The zone closest to the water is submerged when the water level is high. The cushions are filled with mud, sand and lime. No specimens of water bears were found here. *Macrobiotus dispar* and *Hypsibius tetradactyloides* were found only in this sort of habitat. The drifts along the shore might be arranged in Group III also. However it must be noted that drifts are not permanent habitats, they are apt to disappear and gather again through wind and wave action. As to their ecological qualities, they lie between water and terrestrial habitats; they may be characterised by the presence of organic detritus, mud and sand. Two hygrophil forms can be recorded from here, *Macrobiotus macronyx* and *Hypsibius nodosus*.

IV. From the water itself only one species, *Macrobiotus macronyx* was found, in spite of the fact that the abundant plant growth in the margin of the lake, rooted vegetation, filamentous algae, and reed offer conditions favourable to these animals. Samples from the bottom and free waters were looked through also but without positive result. As is known, water bears cannot swim, therefore the specimen recorded from the seston of Lake Balaton (Entz-Kottász-Sebestyén p. 17) was very likely washed into free waters from the shore.

Table I. shows the distribution of Tardigrada according to type of biotop I—IV in the peninsula. *Echiniscus granulatus, Macrobiotus Richtersii, M. Harmsworthi, M. montanus, M. Hufelandi, Hypsibius nodosus, H. Schaudinni, H. convergens, H. Oberhäuseri* and *Milnesium tardigradum* were found in habitribution of all types I—III.

Table II. shows the distribution of Tardigrada according to the various localities in the peninsula (See map).

Pseudechiniscus cornutus, Echiniscus canadensis, Macrobiotus Hufelandi, M. Pullari, M. Harmsworthi, M. montanus, M. furciger, Hypsi-

TABLE I.

Tardigrada fauna of the Tihany peninsula according to type of biotop.

Species	type of biotop											No. of occur.	ecological group
	I. Etxreme sunny dry				II. shadowy humid			III. damp			IV. water		
	1*	2	3	4	5	6	7	8	9	10	11		
1. Echiniscus canadensis *J. Murr.*	+	+	—	+	—	—	—	—	—	—	—	3	xerophil
2. E. bisetosus *Hein.*	—	+	—	—	—	—	—	—	—	—	—	1	xerophil
3. E. granulatus *(Doy.)*	—	—	—	+	—	—	+	—	+	—	—	3	xerophil
4. E. testudo *(Doy.)*	+	+	—	+	—	—	—	—	+	—	—	4	xerophil
5. Pseudechiniscus suillus *(Ehrbg.)*	—	+	—	+	—	—	—	—	—	—	—	2	xerophil
6. P. cornutus *(Richt.)*	—	+	—	+	—	—	—	—	—	—	--	2	xerophil
7. Macrobiotus Richtersii *J. Murr.*	—	+	+	+	—	—	+	+	+	—	—	6	xerophil
8. M. furciger *J. Murr. (?)*	—	—	—	+	—	—	—	—	—	—	—	1	hygr. eurytop
9. M. Harmsworthi *J. Murr.*	—	+	—	—	—	+	—	—	+	—	—	3	eurytop
10. M. montanus *J. Murr.*	+	+	+	+	+	—	+	—	+	—	—	7	eurytop
11. M. Pullari *J. Murr.*	—	+	—	+	—	—	—	—	—	—	—	2	hygrophil
12. M. Hufelandii *S. Schulze*	+	+	+	+	—	+	+	—	+	—	—	7	eurytop
13. M. sp.	—	—	—	—	—	—	—	+	—	—	—	1	?
14. Hypsibius nodosus *(J. Murr.)*	—	—	—	+	—	+	+	—	+	+	—	5	hygrophil
15. H. Schaudinni *Richt.*	—	—	—	+	—	+	—	—	+	—	—	3	hygrophil
16. H. tetradactyloides *(Richt.)*	—	—	—	—	—	—	—	—	+	—	—	1	hygrophil
17. H. annulatus *(J. Murr.)*	—	+	—	+	—	—	—	—	—	—	—	3	hygrophil
18. H. Dujardini *(Doy.)*	—	—	—	—	—	+	—	—	+	—	—	2	hygrophil
19. H. convergens *(Urb.)*	—	—	+	—	—	—	+	—	+	—	—	3	hygrophil
20. H. pallidus *Thulin*	+	—	—	+	—	+	+	—	—	—	—	5	eurytop
21. H. Oberhaeuseri *(Doy.)*	+	+	+	+	+	—	—	—	+	—	—	6	xerophil
22. H. brevipes *Marcus*	—	—	—	+	—	—	—	—	—	—	—	1	xerophil
23. H. Stappersi *(Richt).*	—	+	—	+	—	—	+	—	—	—	—	3	xerophil
24. Milnesium tardigradum *Doy.*	+	+	—	+	+	—	—	—	+	—	—	5	xerophil
25. Macrobiotus dispar *J. Murr. (?)*	—	—	—	—	—	—	—	—	+	—	—	1	hydrophil
26. M. macronyx *Duj.*	—	—	—	—	—	—	—	—	+	+	+**	3	hydrophil
Number of species	7	14	5	18	3	6	8	2	15	2	1		

* 1. roofs; 2. sunny stones and rocks; 3. stone walls; 4. sunny grassy places; 5. tree trunks in woods; 6. stones in the woods; 7. soil in the woods; 8. neighbourhood of spring; 9. stony shore of the lake; 10. drifts on the lake shore; 11. Lake Balaton.

** According to *Daday*.

TABLE II.

Localities on the Tihany peninsula investigated for Tardigrada. (see. map.)

Species	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19 a	19 b	19 c	20	21	22
1. Echiniscus canadensis	—	—	—	—	+	—	—	—	—	+	—	—	—	—	+	+	—	—	—	—	—	—	—	6
2. E. bisetosus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	3
3. E. granulatus	—	—	—	+	—	—	+	—	+	—	—	+	+	—	—	—	—	—	—	—	—	—	—	5
4. E. testudo	+	—	—	+	—	+	+	+	+	+	—	—	—	+	—	+	+	+	+	—	—	—	—	29
5. Pseudechiniscus suillus	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
6. P. cornutus	—	—	—	+	+	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	4
7. Macrobiotus Richtersii	+	+	—	—	—	+	+	+	+	—	+	—	—	—	—	—	+	—	—	—	—	—	—	11
8. M. furciger	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
9. M. Harmsworthi	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	+	—	—	—	7
10. M. montanus	+	—	—	+	—	—	—	—	—	+	+	—	+	+	—	+	+	—	+	+	—	—	—	14
11. M. Pullari	—	—	—	—	+	+	—	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	8
12. M. Hufelandii	+	—	+	+	+	—	+	+	+	+	—	—	+	+	+	+	+	—	+	+	—	—	—	51
13. M. sp.	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
14. M. dispar	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	1
15. M. macronyx	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+**	—	3
16. Hypsibius nodosus	+	—	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	+	—	—	7
17. H. Schaudinni	—	—	+	—	—	+	+	—	—	—	+	—	—	—	—	—	+	+	+	+	—	—	—	9
18. H. tetradactyloides	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	4
19. H. annulatus	—	—	—	—	—	—	—	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	3
20. H. Dujardini	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	+	+	—	—	—	—	3
21. H. convergens	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	4
22. H. pallidus	+	—	—	+	—	—	—	—	—	—	—	—	—	+	+	—	+	+	—	—	—	—	—	6
23. H. Oberhaeuseri	+	—	—	+	—	+	+	+	+	+	—	—	+	+	+	+	+	—	—	—	—	—	—	28
24. H. brevipes	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
25. H. Stappersi	+	—	—	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	—	—	5
27. Milnesium tardigradum	+	—	—	—	—	—	+	+	+	—	—	+	+	+	+	+	+	+	+	—	—	—	—	22
Number of species	13	2	4	7	4	5	7	9	7	5	5	2	5	9	10	6	8	6	8	7	2	1	—	238

* 1. Óvár; 2. Cyprian spring; 3. Calvary; 4. Village; 5. Kiserdő hill; 6. surroundings of Belső-tó pond; 7. Akasztó hegy, Nyársas hegy hills; 8. Aranyház hill and surroundings; 9. Hármashegy hills; 10. Cserhegy hill; 11. Ferry, wave-washed places; 12. Ferry, dry stones; 13. Szarkádi erdő woods; 14. Csúcshegy hill; 15. Nyereghegy hill; 16. Hosszú hegy hill; 17. Bozsai-öböl bay, wave-washed shores; 18. Bozsai-öböl bay, dry shores; 19. shore of Lake Balaton: a. sunny stones, b. wave-washed stones, c. drifts; 20. Lake Balaton; 21. Belső-tó pond; 22. number of habitats.

** according to DADAY

bius tetradactyloides, H. convergens and *Milnesium tardigradum* were found in *Sedum (acre* and *sexangulare (boloniense)* growing in localities No. 1, 3 and 5 (Table II.) also in the rocks of the stone wall in front of the Institute buildings.

Summarizing the results of this faunistical survey of the Tihany peninsula having localities with divers microclimate, it may be said that during the summer months of 1943, 26 species of Tardigrada were found. Most of them are harboured by dry habitats with extreme condition of existence (21). Among these 8 were not found in habitats of other types (differential species). *Echiniscus testudo, Hypsibius Oberhäuseri, S. Stappersi* and *Milnesium tardigradum,* though occurring elsewhere too, here occur most frequently. In shadowy, humid habitats 13 species were found, none of them characteristic of this type of locality. 16 species representing different ecological groups were harboured by damp habitats. There were three differential species found. In the lake itself only one species was collected, which lives also in habitats group III. — *Pseudechiniscus cornutus,* a xerophil species had not previously been recorded from Hungary.

Grateful acknowledgment is made to Docent Dr. O. Sebestyén for translation of the paper.

REFERENCES.

1. Bartoš, E. (1941). *Zool. Jahrb. Syst.* **74,** H. 5/6.
2. Daday, J. (1897). *A magyarországi tavak halainak természetes tápláléka. Budapest.*
3. Entz, G. & Sebestyén O. (1940). *Magyar Biol. Kut. Munk.* **12,** 1—168.
4. Entz, G., Kottász J. & Sebestyén O. (1937). *Magyar Biol. Kut. Munk.* **9,** 1—152.
5. Marcus, E. (1936). *Tardigrada. Das Tierreich,* 66. Lief.

SESTOSOMA VILLOSUM NOV. GEN. ET SP. (CHLOROPHYCEA, PROTOCOCCALES).

By: Dr. Tibor Hortobágyi (Baja, Hungary).

(From the Hungarian Biological Research Institute, Tihany, Lake Balaton.)

With 1 Figure in the text.

(Received for publication 30th November 1946.)

Among the 113 kinds of Balaton seston algae present in the open water at Balaton-Boglár in May, 1943, there was a green alga which did not fit into any genus hitherto described. Because of its autosporadic increase, its small size, oval shape, tiny thorned membrane and individual occurrence there could be no doubt of the family to which it belonged. It had the characteristics of the Oocystaceae family. On the basis of the above-mentioned characteristics it belongs to the sub-family of Oocysteae, though it differs from all genera belonging thereto.

It was distinguished from the *Oocystis* by its thorny cell walls and its lack of gelatinous envelope, from the *Siderocelis* by its wartless membrane, free of ferric-hydroxide, from the *Ecdysichlamys* by its thin membrane thorned to the end and the lack of an extensive gelatinous bed, from the *Nephrocytium* by its single occurrence and also by the lack of the gelatinous envelope; and from the *Kirchneriella* by the shape of its cell, its ornamental membrane and its single non-gelatinous appearance (B. Fott, 1934). (Fig. 1—12).

From this it is apparent that the new alga can be established in the Oocystea sub-family of the Oocystacea only by the creation of a new genus, as below.

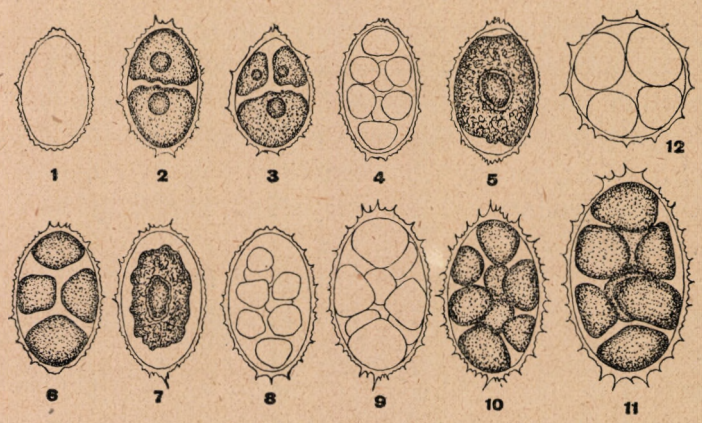
Sestosoma nov. gen.

The cells are oval-shaped and their membranes ornamented by larger or smaller fine thorn-like formations. The cell wall is thin and colourless. The cell is almost completely filled by its chromatophore;

in the middle of it is a large pyrenoid. They increase by 2—4, more usually, however, by 8 autospores. They live singly and have no gelatinous envelopes.

Sestosoma villosum nov. sp.

The cells live singly, usually as expanded oval shapes, very rarely slightly depressed; always completely symmetrical. Their cross-section is a true circle. They are 8—14 μ in length, 4.8—8.8 μ in width, but



Figures 1—12: Sestosoma villosum. Figs. 1—11. Specimens of different sizes and ages. Figure 12: Cross section of plant in Figure 11. Magnified 1450 x.

most of them vary between 8—9.2 x 4.8—6.8 μ in dimension. On the thin and colourless surface of the cell can be seen thorns of differing sizes, irregularly disposed, not exceeding 1 μ. These thorns are sometimes a little bigger at the end of the cells and form a small thorny crown. Here the cell wall is also somewhat thicker. Now and then, exceptionally, a cell can be found on which the thorn-like formations are lacking and the cell wall is merely irregular in surface. Others are seen whose membranes are almost smooth, or where the stronger thorns are lacking, of where these formations exist only on one side. These are generally younger specimens.

The cell is almost completely filled with a large, fine green chloroplast, in the midst of which is an equally powerful pyrenoid. Propagation takes place by 2,4, 8 autospores. The specimens with 8 autospores are especially common. The autospores are light green, their pyrenoids not at all, or only just, visible. They are closely packed in the mother cell. I could not succeed in demonstrating a definite mucus covering with gentian violet or methylene blue, but in dilute Chinese ink it seemed as if a thin, colourless layer could be discerned.

In respect to its systematical position I place it in the order of the Chlorophyceae Protococcales, in the Oocysteae sub-family of the Oocystacea, between the genera *Ecdysichlamys* and *Siderocelis*. It differs from the genus *Ecdysichlamys* in that:

1. Its cell membrane is thin. 2. The cells are not in an extended gelatinous bed. 3. Its form is a regular oval shape. 4. Its whole surface is covered with fine thorns, its ends with a thorny crown. 5. It lives in seston.

It differs from the genus *Siderocelis* in that:

1. Its membrane is not warty. 2. It contains no ferric-hydroxide.

Its relation towards the genus *Ecdysichlamys* is indicated by the 1—1 tiny thorn found on the ends of the cells of the *Ecdysichlamys obliqua* G. S. West, and by the somewhat lengthened, oval-shaped, bipolar, not quite symmetrical cell shape and, lastly, by its almost identical dimensions.

It is related to the genus *Siderocelis* as shown by the shape of its cell, thin membrane, dimensions and by the circumstances in which it occurs.

I observed it only in May. It seems to be an organism appearing suddenly and propagating rapidly, not appearing in the seston for a long time.

As to its sociological relations we get the following picture:

Exclusive of Diatoms,

Cyanophyceae	19
Flagellatae	13
Dinoflagellatae	5
Chlorophyceae	67
Conjugatae	8
Together	113

Quantitatively the single groups took part in the phytobiocoenosis in the following percentual proportions:

Cyanophyceae	12.7%
Flagellatae	0.1
Dinoflagellatae	1.8
Chlorophyceae	16.4
Conjugatae	1.5
Heterocontae	9.0
Diatomeae	58.5
Total	100%

Among the Diatoms there are many with empty valves. These were carried among the plants of the seston by the waves caused by the nearly constant wind.

The ten most common algae of the biocoenosis are:

1.	Planktonema Lauterborni	9.0%
2.	Gomphosphaeria lacustris	7.3
3.	Microcystis holsatica	4.3
4.	Sestosoma villosum	2.6
5.	Scendesmus intermedius	2.4
6.	Oocystis submarina	2.0
7.	Ceratium hirundinella	1.6
8.	Dictyosphaerium pulchellum	1.4
9.	Tetrastrum staurogeniaeforme	1.4
10.	Staurastrum contortum	1.1
	Total	33.1%

These algae form 33.1% of the plants of the biocoenosis. The new plant, which occupies the 4th place in the phytobiocoenosis, was the most common among the Chlorophyceae. It makes up 15.8% of the entire quantity of green algae.

The temperature of the water during the collections was 17.5° C, the pH 8.4. I made the collections with a net of miller gauze No. 25.

Grateful acknowledgment is made to Mrs. Thompson Vass for the English translation.

REFERENCES.

Brunnthaller, J. (1915). *Protococcales. In Paschers Süsswasser-Flora H.* 5. *Jena.*

Fott, B. (1934). *Siderocelis, eine neue Gattung der Protococcalen. Beih. z. Bot. Centralblatt Bd.* LII. Abt. B. 112—118.

QUANTITATIVE METHODS IN COLLECTING GRASSHOPPERS.

By: JÁNOS BALOGH.

(From the Hungarian Biological Research Institute, Tihany, Lake Balaton.)

One of the principal duties of quantitative biosociology is to establish the exact numerical abundance of animals on a given territory. In hydrobiology and phytosociology several quantitative methods are in use, but in terrestrial biocenology not many are known. The collection and counting of terrestrial animals, principally the Arthropoda, also involve many technical difficulties not easy of solution. Most methods so far have been merely "quasi quantitative" (BLAKE, 1926), as the quantitative results obtained were unreliable. A wider knowledge of these methods can be got from P. Palmgren's "Quantitative Untersuchungen über die Vogelfauna in den Wäldern Südfinnlands" (p. 46—54), and will therefore not be dealt with more fully here.

To eliminate some of the faults of these methods, I worked out a new one by which the density of grasshoppers can be established with complete exactitude. I called the method the rectangular-band method (German Quadratbandmethode), as it really consists in a transition between the linear appraisement (Linientaxierung) and the netted square methods (Netzquadratmethode). The new method, as will appear below, is extraordinarily simple, the counting can be done quickly, and the results thereby obtained are very exact, that is to say, absolute quantitative values.

For the survey I used a rectangular band outlined in light-coloured twine, consisting of 10 successive squares 1x1 m in dimension. This outline I stretched out on the area under investigation, directly on the ground, by means of iron or wooden pegs, and then left the place for 5–10 minutes. This is necessary for the grasshoppers disturbed by these preparations to grow quiet again and the designated territory to acquire its original grasshopper population once more. When the 5—10 minutes had elapsed I went circumspectly back to the rectangular band and looked through the squares, one after the other. During the examination the grasshoppers jumped from the squares, so that I had

only to count the animals which jumped out and note their number separately for each square.

The work of counting naturally demands great attentiveness. In the first place, care must be taken to determine whether the grasshoppers which jumped were inside or beyond the limits of the square, so as not to count the animals outside it. Observation must be made too of the animals which jump from the square under consideration into the next one, not yet examined. In this case the animal or animals which have jumped out must be left out of consideration. Finally, note must be taken of the more easily frightened, more lively or more agile grasshoppers which may jump out too soon on our approach, even before one had begun the examination of their square.

This apparently complicated task can be accurately accomplished if a small-scale drawing of the netted rectangle (that is, 10 squares drawn in a line) is kept in the hand, with a pencil, and the numbers of grasshoppers noted, not only those that jump out from the square under immediate examination, but from the following squares as well. The more of these bands we can examine at a time during the investigation of a biocenosis, possibly 3—5, the more the results will approach the ideal condition. In this way, by counting 30—50 m^2 we can calculate the average sample/m^2, with accurate results.

With this method, however, only the abundance of the grasshoppers can be established, as it is often impossible to determine the species of the jumping animals. So the composition of the grasshopper population as to species must be determined indirectly. This is done by catching any considerable number, if possible more than 100, of grasshoppers on the same field, carefully avoiding any unintentional selection and actually catching every animal at random. The identification can be made at home and from it percentual frequencies of the species established.

The rectangular band method can be carried out by one person and faultlessly if the grasshopper abundance in the area is not greater than 2—6 specimens per m^2. If it is greater, then 2 persons can manage it, by advancing closely one after the other, each counting the animals jumping from half a square (50x100 cm^2) and at the end putting their results together. The following record was made with this system.

Fumana vulgaris — Festuca vaginata — Cladonia faliacea — Magyarica sociation, Szigetmonostor, 7. VII. 1946:

**

Band N° I.	11	13	9	6	9	7	10	11	8	10
„ „ II.	9	11	12	8	7	11	7	10	11	12
„ „ III.	10	11	9	8	4	12	10	7	7	8
„ „ IV.	9	9	11	12	8	8	7	10	9	9
„ „ V.	11	10	8	7	7	9	10	8	4	6

As we see, from the 9/m² value, the maximum deviation varies between +0.8 and —1.0, therefore the limit of error on the basis of 10 squares is about 10%. Counted on the basis of the first 2 bands (that is 20 squares) the average value is 9.6/m², the difference being therefore +0.6; on the basis of 30 squares 9.1/m², the difference therefore +0.1; on the basis of 40 squares 9.2/m², the difference being +0.2. With the increase in rectangular bands therefore we continually draw closer to the ideal average value. According to my experience, with 3—5 rectangular bands, that is, with the examination of 30—50 square metres, the values are practically exact, so that it is not worth while investigating any larger number. The survey of 3—5 such rectangular bands together with their preparation takes about 15—30 minutes, so that though the work is very exact it goes very fast.

With this method in the past 3 years I have counted the grasshopper populations of many phytocenoses and obtained extremely valuable results. If the numerical results are combined with the weight, that is, if the average weight of the species is established, we arrive at an exact estimation of the weight of the grasshopper population of the phytocenosis on a given territory (as for example 1 ha.). Such investigations give a very instructive picture of the significance of the grasshopper population of the phytocenosis in question and if the surveys are carried out several times between spring and autumn, of the fluctuations in the entire population as to weight and numbers.

Grateful acknowledgment is made to Mrs. J. Thompson Vass for the English translation.

REFERENCES.

Blake J. H. 1926. A comparison of the animal communities of coniferous and deciduous forests. *Illinois Biol. Monogr.* **10.** 148.

Palmgren P. 1930. Quantitative Untersuchungen über die Vogelfauna in den Wäldern Südfinnlands. *Acta Zool. Fenn.* **7.** 209.

BERICHT DER ORNITHOLOGISCHEN BALATON-FORSCHUNG IN DEN JAHREN 1942 UND 1943.

Von: ANDRÁS KEVE, IMRE PÁTKAI, MIKLÓS VON ÚDVARDY, ALBERT VERTSE (Budapest).

(Aus dem kgl. Ungarischen Ornithologischen Institut und aus dem Ungarischen Biologischen Forschungsinstitut).

(Eingegangen am 1. November 1944.)

Die systematische ornithologische Durchforschung des Balaton-Gegendes trat in sein zweites und drittes Jahr, leider ohne einer offiziellen Auftrag, doch gelang es uns zur Lösung der dringendsten Aufgaben Zeit zu gewinnen. Unsere Beobachtungszeiten verteilten sich folgendermassen: 1942: KEVE 12.—18. I., 2.—7. IV., 23.—26. V., 7. VIII. — 24. IX.; PÁTKAI 11.—21. VI., 17.—22. XII.; VERTSE 1.—10. I., 5.—12. V. — 1943: KEVE 27. VII.—3. VIII., 23. VIII.—20. IX., v. UDVARDY 16. VIII.—5. IX., 27. XII.—2. I. 1944. Insgesamt verweilten wir in 1942 74, in 1943 51 Tage am Balaton. Die wichtigsten zwei Resultate unserer Beobachtungen sind, dass wir in Brutzeit in weiterem Umkreis die Umbgebung des Sees überblicken konnten, besonders den Wald bei Keszthely (Vállus, Rezi), und dass wir im September möglichst unser ganzes Interesse dem Strande von Balatonzamárdi zuwenden konnten. Im Dezember machten wir eine Sammelfahrt für Grossmöven, sowie für Enten-Beobachtungen. Wir kontrollierten die Sammelresultate von GASZTON GAAL von GYULA und stellten fest, dass nur einige Fundorte und Daten in der Litteratur nicht völlig stimmen.*

Als neue Arten für dem Balaton wurden in diesen Jahren: *Lanius senator, Oidemia nigra* und *Chlidonias hybrida* gefunden, die Liste muss ausser dem durch *Branta ruficollis* und *Numenius phaeopus* ergänzt

* Wir sprechen unseren innigsten Dank dem Ung. Biol. Forschungsinstitut aus für eine verständige Untersützung, und ebenso allen jenen Herrn, die die Bedeutung der Idee von Prof. dr. GÉZA ENTZ (†) richtig aufgefasst haben und sich beeilten ihre Angaben uns mitzuteilen, namentlich: DR. F. V. BARANYOVITS, DR. T. CSÖRGEY, DR. B. V. DARNAY-DORNYAY, DR. H. DORNING, G. V. HERTELENDY, S. HOFFMANN, DR. O. KELLER, L. V. NEMESTOTHY, DR. L. E. V. SZALAY, V. SZÉKELYHIDY, DR. M. VASVÁRI (†). J. VÖNÖCZKY-SCHENK (†).

werden und durch die mit Sicherheit aufzunehmenden Arten der Sammlung von GAAL: *Calidris canutus, Limosa lapponica, Larus melanocephalus,* kann die Zahl der Arten der Balaton-Gegend auf 268 erhöbt werden. Wir beobachteten in 1942 die folgende Arten, die wir im Jahre 1941 nicht gesehen haben:

(in der Brutzeit:) *Muscicapa p. parva* BECHST. (Vállus), *Locustella n. naevia* BODD. (Tihany, Tördemic-Ederics, Kisbalaton, Balatonmária), *Acrocephalus palustris* BECHST. (Tihany), *Monticola saxatilis* L. (Lesencenémetfalu), *Aquila p. pomarina* BREHM (Vállus), *Platalea l. leucorodia* L. (Kisbalaton); am Frühlings- und Herbstzuge: *Pandion h. haliaëtus* L. (Tihany, Zamárdi), *Calidris testacea* PALL. (Zamárdi), *Calidris minuta* LEISL. (Balatonöszöd), *Calidris c. canutus* L. (Zamárdi), *Chlidonias h. hybrida* PALL. (Zamárdi). — Im Jahre 1943 nur ein Durchzügler bzw. Wintergast: *Oidemia nigra* L.

JÄHRLICHER ÜBERBLICK. 1942.

I. 1942 Siehe KEVE-PÁTKAI-VERTSE 1943, p. 177—185.

2.—7. IV. 1942. Durch dem langen Winter ist die Natur noch ganz zurückgeblieben und hat noch kaum den vorjährigen Mittelmärzzustand erreicht. Die Wetterlage ist sehr wächselnd, überwiegend trüb und regnerisch. Der Balaton ist stürmisch und hat einem hochen Wasserstand. Der Vogelleben entspricht in Grossen-Ganzen dem vorjährigen nur in mancher Hinsicht ist es zurückgeblieben. Am Balaton schwimmen überall Schwärme von *Reiherenten,* heuer gab es aber auch noch *Schellenten, Gänse-* und *Zwergsäger. Haubentaucher, Lach-, Sturm-* und *Silbermöven* findet man auch überall am See. Die Aszófőer Ecke war ebenfalls so gut bevölkert wie vor einem Jahr. Die Kleinvögel zeigen sich sehr spärlich *(Zilpzalp, Mönchgrasmücke, Hausrotschwanz, Heckenbraunelle, Steinschmätzer, Rotkehlchen* usw.) Die Wälder und Büsche sind durch den Zug der Drossel karakterisiert *(Amsel, Sing-,* und *Weindrossel,* heuer auch *Wacholder-* und *Misteldrossel).* Die *Rauchschwalben* zeigten sich in anderen Jahren zu dieser Zeit regelmässiger und in grösserer Anzahl. Dagegen kamen *Flussuferlaufer, Flusseeschwalbe, Rohrschwirl* früher als im vorigen Jahre an, und heuer haben wir sogar den *Fischadler* in der Aszófőer Ecke am Frühlingszuge erwischt.

5.—12. V. 1942. Balatonberény. Nach lange dauernden Regen und kaltem Wetter trat plötzlich sommerliche Wärme ein. Die Vögel beginnen in erhöchtem Tempo zu brüten. Die Wasserornis zeigt sich nur spärlich weil hier der Schilfrand zu schmal, deswegen der Wellenschlag

zu kräftig ist. Ein grösseres Schilfgebiet befindet sich nur vor Balatonmária in der Richtung des Einflusses der Zala; die hier ständig anwesende Arten sind: *Stockente, Rohrdrossel, Teichrohrsänger,* 2—3 *Flussuferläufer.* Für das Ufer von Balatonberény ist karakteristisch, dass am Rande plötzlich abfällt, aber die durch dem Wellenschlag abgerissenen Lehmplatten liegen flach vor dem Abhang. Auf diese Platten liefen die *Flussuferläufer* gegen die Wellen bis zum Bauch im Wasser, griffen ihre Beute heraus und liefen schnell wieder zurück oder liessen sich von den Wellen auf Ufer werfen. Manchmall war eine *Lachmöve,* ein *Haubentaucher,* ein *Moorenten-Paar* und 4 *Flussseeschwalben* zu sehen. Vor einem Gewitter erschienen über Balatonberény zahlreiche *Mauersegler*-Flüge, am 9. V. sogar über 100 Stück. Sie kamen von der Richtung von Keszthely; beim gutem Wetter waren kaum 4—5 Exemplare zu sehen. Erwähnenswert ist, dass in den hohen Lösslehnen 2—3 Paar *Turmfalken* gebrütet haben, einmal erschien hier auch ein *Brauner Milan* und manchmal eine *Blauracke.*

23.—26. V. 1942. Während dieser kurzen Beobachtungsperiode herrschte schönes Wetter, nur am 24. Abends kam ein plötzlicher Sturm. Die Natur hat ihren normalen Entwicklungsstand schon erreicht, nur die Nutzpflanzen sind noch etwas zurücklieben. Der Wasserstand ist sehr hoch, die Tümpel sind voll mit Wasser. Das Vogelleben entspricht dem vorjährigen. Die Vögel haben sich vom Balatonsee schon zerstreut, nur *Haubentaucher* schwimmen noch dort (1—2, höchst 6). Interessant ist es, dass wir den *Mittelsäger* heuer ebenfalls im Mai angetroffen haben. Es scheint, dass der Wind im Belsősee an der Brüten wieder grosse Schaden verursacht hat. Wir fanden mehrere vom Wind zerrissenen *Rohrdrossel*-Nester, vom *Blässhuhn* nur ein frisches Nest ohne Eier, dagegen scheint die Brut der *Haubentaucher* ziemlich gut gelungen zu sein, die Jungen schlüpften eben jetzt aus. Der *Storch* ist im Dorfe an sein altes Nest zurück gekehrt.

11.—21. VI. 1942. Der Himmel war immer bewölkt mit häufigem Regen. Ich bin um den Balaton herum gefahren und widmete mein besondere Interesse den Weinbergen und grossen Waldungen am Nordwest-Ufer. In Tihany machte ich Vergleiche mit dem vorigen Jahr: Die Juni-Ornis der Halbinsel ist reicher geworden durch die *Schwanzmeise,* den *Heuschreckenrohrsänger* und den *Grünspecht.* Auffallend war der völlige Mangel des *Wiedehopfs,* der voriges Jahr so häufig war. Sonst traf ich in der Balaton-Gegend den *Wiedehopf* auch nur an zwei Stellen an, was ebenfalls für eine bedeutende Abnahme spricht. Die Zahl der *Haubentaucher* hat auch abgenommen. Kann sein, dass die

Ursache eine sehr früh gelungene Brut war, infolge dass die Vögel im Juni sich schon zerstreut haben. Auch eine übersommernde *Reiherente* traf ich noch an.

Erwähnenswerte Arten der anderen Stellen der Nordwestküste sind: Auf den steinigen Wiesen zwischen Akali und Zánka traf ich die *Heidelerche* und mehrere Familien des *Steinschmätzers* an. In den Wäldern von Badacsony sind alle drei *Laubsänger*-Arten *(Zilpzalp, Fitis* und *Waldlaubsänger)* vorgekommen. Die *Amsel* und *Singdrossel,* die beim Balaton gar nicht häufig sind, zeigten sich hier in schöner Anzahl. Bei Lesencenémetfalu sah ich eine *Heckenbraunelle,* ebenda das Nest eines *Steinrötels* in einer Ventillationsöffnung eines Weinkellers. Die Vögel des alten Buchen- und Eichenwaldes waren: *Zwergfliegenschnäpper, Schwarzspecht, Zwergohreule* und *Hohltaube.*

Am 15. VI. sah ich schon den ersten Flug junger *Star*en, und beobachtete während dieser Zeitperiode die Fütterung oder das Führen von Jungen der folgenden Arten: *Elster, Star, Kernbeisser, Grünling, Buchfink, Brachpieper, Baumpieper, Bachstelze, Kleiber, Kohlmeise, Sumpfmeise, Schwanzmeise, Halsbandfliegenschnäpper, Zwergfliegenschnäpper, Rohrdrossel, Singdrossel, Amsel, Steinrötel, Steinschmätzer, Braunkehlchen, Schwarzkehlchen, Grünspecht, Waldohreule, Turmfalke, Rohrweihe, Storch, Firschreiher, Purpurreiher, Sumpfreiher, Nachtreiher, Zwergrohrdrommel, Stockente, Knäckente, Haubentaucher, Hohltaube, Ringeltaube.*

14.—16. VIII. 1942. Tihany. Die drei Augusttage sind bei schönstem Sommerwetter abgelaufen. Der Zug der Kleinvögel *(Zilpzalp, Fitis, Mönch-, Zaungrasmücke, grauer Fliegenschnäpper* usw.) hat schon begonnen, da sich aber alles noch ganz im Anfangsstadium befindet, herrscht noch Sommerstille. Am Morgen singt noch die *Nachtigall.* In der Somoser Bucht erschienen die jungen *Silbermöwen.*

7.—24. IX. 1942. Tihany. Diese längere Herbstbeobachtungszeit opferten wir hauptsächlich den Beobachtungen der Strandvögel. Die Wetterlage war die günstigste. Zwischen 7.—16. IX. war an den Beobachtungen auch Kollege Claude Thibaut de Maisieres (Bruxelles) beteiligt. Der Zug verglichen mit 1941 im allgemeinen quantitativ ärmer, aber qualitativ reicher war. Wir besuchten auch jenen Strandteil zwischen Balatonszemes und -Lelle, wo vor 50 Jahren G. von Gaal gearbeitet hat. Diese Stelle ist auch heute noch viel günstiger als andere, weil sie ungestörter und weiter ist. Es gab hier ungeheure Massen von *Enten,* ausserdem in grosser Zahl *Alpenstrandläufer, Lach-* und *Silbermöwen, Flussseeschwalben, Trauerseeschwalben* und *Sandregen-*

pfeifer. (20. IX.) Bei Balatonzamárdi sah man *Alpenstrandläufer* in 1—2 Exemplaren beinahe jeden Tag, nachher war der *Kiebitz* und die *Bekassine* am regelmässigsten, dann *Sandregenpfeifer* und *heller Wasserläufer* und vereinzelt *islandischer Strandläufer, bogenschnäbeliger Strandläufer, Flussuferläufer, Kampfläufer, dunkler Wasserläufer, Brachwasserläufer, grosser Brachvogel.* Besonders abwechslungsreich war das Möwenleben: *Lachmöwe, Silbermöwe, Weissbärtige Seeschwalbe, Schmarotzer-Raubmöwe, Flussseeschwalbe, Sturmmöwe, Zwergmöwe.* Der Wasserstand des Balaton war noch immer sehr hoch, was für die Enten sehr günstig gewesen wäre, doch waren sie nicht in solcher Masse anzutreffen wie im vergangenen Jahr. In diesem Jahr war die herrschende Art die *Stockente,* die *Schnatterente* wie die *Krickente* bleiben weit hinter ihr; oft zeigen sich *Spiess-* und *Pfeifenten,* die voriges Jahr völlig fehlten und es gab auch *Löffelenten, Tafelenten, Knäckenten* und *Moorenten,* auch die *Reiherente* kam zu früh hierher, am 14. IX. Manchmal schwammen einige *Graugänse* herum und am 23. IX. kamen die *Saatgänse* an. Den *Fischadler* erwischten wir hier am Herbstzuge. *Reiher (Fisch-, Purpur-, Edelreiher)* zeigten sich wieder sehr spärlich. In den gemischten *Schwalben*flügen gab es auffallend spät (15. IX.) noch einen *Mauersegler.* Wir sahen viel *Eisvögel* und endlich jagten die *Baumfalken* regelmässig auf dem Strande und liessen sich auf den Sandbanken nieder.

Die Aszófőer Ecke war ornithologisch nicht so günstig. In der Somoser Bucht, so wie in der Bucht der Biologischen Institut fangen die *Haubentaucher* und *Lachmöwen* heuer schon jetzt sich zu sammeln. Das Vogelleben der Wälder und Gebüschen war viel ärmer als im Jahre 1941, der Zug der *Baumpieper* fiel aber auf. Zusammengefasst war das herbstliche Vogelleben scheinbar ärmer, aber abwechslungsreiher als im vorigen Jahr, es schien etwas früher einzusetzen, mit Ausnahme des verspäteten *Mauerseglers.*

17.—22. XII. 1942. Tihany. Wetter: windstill, laue Witterung (—1, —2°C), Regen, morgens Nebel. Auf der Balaton auffallend wenig *Tauchenten,* nur in kleinen Truppen (5—6 St.) *Reiherente* und *Schellente,* während sie im vorigen Jahre schon am Ende Dezember in 100 köpfigen Scharen vorhanden waren. Hingegen schwamm die *Stockente* in verhältnissmässig grosser Zahl, in Truppen von 50—60 Stücken bei den Röhrichten. Es war auffallend die grosse Zahl der *Möwen: Lachmöwe* etwa 3000 St., *Silbermöwen* in etwa 600 Stücken waren in der Tihany—Szántóder Enge. Der Ruf der Silbermöwen war in der ganzen Nacht

vernehmbar. Die *Elstern* schliefen truppenmässig in der Röhricht, auf meinen Schuss flogen etwa 60 Stücke auf. Bemerkenswertere Arten am 18. XII.: *Eisenten* 2 St., *Prachttaucher* 1, in der Somoser-Bucht; weiters *Heringsmöwe* 6 ausgefärbte St., *Sturmmöwe* 15—20, *Zwergsteissfuss* 2 St., am 19. XII.: *Bachstelze* 1, *Wasserpieper* 1, *Wanderfalke* 1, *Girlitz* 5. Am 20. XII.: *Prachttaucher* 2, *Merlinfalk* 1, *Sperber* 1 ♀, *Eisente* 1, *Bergente* 4 St. Am 21. XII. bei Örvényes *Reiherente* 100, *Silbermöwe* 150. Nach den Beobachtungen der Fischer fangen die Grossmöwen nur die *Sichling (Pelecus cultratus)* und fressen sie keinen anderen Fisch.

1943.

27. VII.—3. VIII. 1943. Tihany. Grosse Hitze mit beinahe völligen Windstille, nur am 28-ten ein kurzes Regen. Der Wasserstand war hoch, auch der von der tihanyer Belső-tó (Innerer Teich); das Schilf ist daher fast ganz ausgestorben. Der Boden der Halbinsel war steinhart, doch die Vegetation frisch und dadurch auch das Vogelleben rege. Drei Beobachtungen müssen extra erwähnt werden: am 28. VII. zwei *Kreuzschnäbel* über Balatonföldvár — im Jahre 1941 hatte PÁTKAI zur selben Zeit sie in Balatonfüred beobachtet. Dann brüteten *Bienenfresser* in einer ganz kleinen Lösswand neben der Landstrasse. Die *Silbermöwen,* meis alte Exemplare zeigten sich auch im Juli. Die Reife des Holunders verursachte den Beginn des Zuges der *Grasmücken* anfangs August. Die *Rauchschwalben* scharten sicht schon am 31. VII. zusammen.

16. VIII.—20. IX. 1943. Tihany. Mitte August und Mitte September herrschte eine sommerliche Hitze, kühl und abwächselnd war das Wetter zur Zeit des Monatwechsels. Der Holunder lockt Mengen von Singvögeln an: besonders *Grasmücken, Drosseln, Nachtigallen;* auffallend viele *Pirole* im Verhältnis zu anderen Jahren. Der Zug der *Fliegenschnäpper* war zur Monatswende am stärksten. Die *Laubsänger* waren während der ganzen Zeit zu sehen: *Fitis-* und *Weidenlaubsänger* in gleicher Zahl, *Waldlaubsänger* bedeutend weniger. *Schwanzmeisen* zeigten sich regelmässig im Wald des Abtei-Hügels. Wie im vorigen Jahren, haben wir auch zu dieser Zeit den *Grauspecht* beobachtet, diesmal im Szarkáder Wald. Die Kleinvögel dieses Waldes besuchten regelmässig die nebenanliegenden Schilfwälder, sogar den *Kleiber* trafen wir im Schilf an. Der *Kreuzschnabel* zeigte sich öfters über den Halbinsel und in Balatonföldvár. *Goldhähnchen* und *Tannenmeisen* zeigten sich besonders früh auf den Weg, Mitte IX. zeigten sie sich nur

sporadisch. Es mag noch erwähnt werden, dass abends am 19. VIII. ein Flug *Weisstörche* dem Ufer der Halbinsel folgend über den Szarkáderwald in NW—O Richtung flog.

Das Wasservogelleben zeigte schon mehr Abweichungen vom vorjährigen, was dadurch entstanden ist, dass der Balaton wegen der Bauten am Sió-Kanal im Herbst dieses Jahres nicht abgelassen wurde. So blieb der Wasserstand auch im Herbst so hoch, dass der Wasserspiegel die Grasbänke überall erreichte und kaum etwas vom Strand-Ufer trockenblieb. Natürlich fanden so die durchziehende Strandvögel keine günstige Stelle, wo sie sich niederlassen konnten. Einzelne Stücke, die wir durch Zufall im Fluge erwischten, zeigten aber, dass der Zug doch vor sich ging. Eine andere Abweichung war die auffallend frühe Ankunft der nordischen Schwimmvogelgäste. Die *Reiherenten* erschienen zu Hunderten schon Mitte September auf den freien Wasserspiegel. — Im Jahre 1941 beobachteten wir am 8. X., im J. 1942 waren kleine Flüge am 14. IX. zu sehen. Die Ankunft der *Zwergmöwen* stimmt mit dem vorjährigen überein (18. IX.). Die *Lachmöwen* und *Taucher* scharten sich wieder an gewissen Stellen in engen Truppen zusammen. Es ist das also eine regelmässige Erscheinung, die dieses Jahr sehr früh, schon am 9. IX. eingetreten ist — im Jahre 1941 wurde sie am 10. X., im Jahre 1942 am 21. IX. beobachtet. — Die Aufgabe der zukünftigen Forschung wäre es, diese wiederkehrenden Periodizitäten im Wasservogelleben, sowie ihre Ursachen zu untersuchen, was für ganz Mitteleuropa von Wichtigkeit wäre.

Am Belső tó war die herrschende Entenart die *Schnatterente*. Hunderte von *Blässhühner* überfielen den kleinen See. Von Mitte VIII. bis Anfang IX. wuchs ihre Zahl noch immer, und noch Anfang IX. fanden wir halbausgewachsene Junge. Der Külső tó (Wiese) war im Sommer nur etwas feucht und wir konnten die Ansammlung der *Nebelkrähen* und *Tauben* dort wieder feststellen. In der zweiten Hälfte des Septembers zogen die gewöhnlichen *Krähen*züge wieder dem Ufer entlang.

27. XII.—2. I. 1944. Tihany. Von Anfang Dezember an zeigten sich Fröste. Während der Beobachtung herrschte ein klares, sonniges, aber windiges Wetter. In der Nacht fiel die Temperatur auf —2—3°C. Das Vogelleben war ganz winterlich. Die Kleinvögel scharten sich an den Wäldern und Gebüschen zusammen — *Meisen, Goldhähnchen, Gimpel, Bergfinken, Seidenschwänze, Wacholderdrosseln* waren die vorherrschenden Arten. Sonst sind aber die Wälder still. Die Wiesen wurden ausser von *Corviden* nicht besucht. Wasservogelleben fand man nur am weiten freien Wasserspiegel des Balatons. Im Schilf erschienen nur ver-

einzelte *Rohrammern,* einige *Haubentaucher* und *Säger* und die *Meisen*-Flüge.

Der freie Wasserspiegel des Balaton ist stellenweise sehr belebt. Die *Reiherente* ist weitaus am zahlreichesten, besonders in der Umgebung der Reede in lockeren Scharen. Auch cca. 4—500 *Stockenten* hielten sich bei der Halbinsel auf. Die Bergenten mischten sich unter die Flüge der *Reiherenten. Schellenten* erschienen erst Ende Dezember in selbstständigen Trupps, sonst waren sie auch die *Reiherenten*-Flügen beigemischt. Von anderen sahen wir (nach ihrer Häufigkeit geordnet) *Blässhühner, Zwergsäger, Haubentaucher, Samtenten, Prachttaucher, Zwergsteissfüsse, Eisenten, Trauerenten, Gänsesäger.* Flüge von *Lachmöwen* schwommen nur zwischen der Reede und der Fischerkolonie, sonst nur vereinzelte Exemplare, wie auch eine *Silbermöwe* weit draussen am See beobachten konnte.

SYSTEMATISCHER TEIL.

An dieser Stelle erwähnen wir nur die wichtigsten Beobachtungen.

Nucifraga c. caryocatactes L. — S. Hoffmann sandte ein ♂ von Keszthely am 20. XI. 1943.

Loxia c. curvirostra L. — Zwischen 28. VII. und 19. IX. 1943. mehrfach beobachtet besonders in Balatonföldvár, aber auch in Tihany. Am 26. VIII. konnten wir längere Zeit beobachten wie 5 Exemplare mit Kohlmeisen die Samen der Sonnenblumen auf einem Feld in Tihany auszupften. 20 Stück beobachtete Vönöczky noch am 9. XII. bei Balatonrendes.

Parus a. ater L. — erschien im Jahre 1943 sehr früh (17. IX.).

Regulus r. regulus L. — war schon am 4. IX. 1943. in Tihany.

Lanius s. senator L. — Endlich gelang L. v. Nemestóthy im Jahre 1942. nicht nur sein Vorkommen, sondern sogar sein Brüten in der Balaton-Gegend festzustellen, als er am 20. VII. 4 Junge bei Zalacsány-Felsőmándpuszta beringen konnte.

Bombycilla g. garrulus L. — 50—60 St. zwischen 27. XII. 1943—2. I. 1944.

Merops a. apiaster L. — Ein Paar brütete auf der Halbinsel im 1943. Sie fütterten noch am 1. VIII., besonders mit Hummeln und manchmal mit grossen Libellen. Am 27. VIII. beobachteten wir einen grossen Flug von mehreren Hundert Stücken, die über einem Luzernefeld wie Schwalben herumflogen — es waren auch einige *Rauchschwalben* unter ihnen. Wahrscheinlich jagten sie die kleine *Schmetterlinge,*

die die Luzerne massenhaft besuchten. Manche Bienenfresser hüpften am Boden, wahrscheinlich auf *Heuschrecken* jagend. Nach einer Woche (3. IX.) fand ich den Flug etwas an Zahl abgenommen an derselben Stellen.

Gyps f. fulvus Habl. — Nach dem Bericht von G. v. Hertelendy hat man 26. V. 1942. 16 Stücke, darunter 3 dunkle Junge in dem sogenannten „Dobogó" Karstgebiet zwischen Zalahaláp, Sáska und Diszel beobachtet.

Cygnus cygnus L. — Dr. B. v. Darnay-Dornyay und Prof. Dr. Keller teilten mit, dass im Februar 1943 zwei Singschwäne sich im Kanal der heissen Quellen von Héviz niederliessen und beide erlegt wurden.

Branta ruficollis Pall. — In unserer Faunenliste vom 1941 blieb diese Art aus. M. Vasvári hatte die Güte uns auf die Lücke aufmerksam zu machen, dass im Zoo von Budapest in 1943 lebende Exemplar bei Balatonszabadi im Herbst 1934 verletzt wurde.

Clangula hyemalis L. — konnten wir am 1. I. 1944 wieder feststellen (5 St.).

Oidemia f. fusca L. — Zwischen 27. XII. — 2. I. 1944 konnte ich fast täglich je 2 Samtenten beobachten, immer nur ausgefärbte ♂♂.

Oidemia n. nigra L. — Am 28. XII. 1943 stellte ich auch die Trauerente in den *Reiherenten*-Scharen an der Reede fest. Neue Art für dem Balaton!

Charadrius morinellus L. — Dr. H. Dorning hatte die Güte uns aufmerksam zu machen, dass diese Art in unserer Hauptbericht vom Jahre 1941 ausblieb. (Zool. Lapok, 1901. p. 361—362.). Leider war das ausgestellte Exemplar zu unverlässlich, dass es tatsächlich aus der Balaton-Gegend stammt.

Charadrius temmincki Leisl. — siehe bei Charadrius morinellus.

Calidris c. canutus L. — Am Strande von Balatonzamárdi erschien einer am 15. IX., nach zwei Tagen gelang es auch einen zu sammeln. Er hat sich hauptsächlich in der Gesellschaft eines Alpenstrandläufers im Prachtkleide gehalten. Der Balg ist der 13-te aus Ungarn, und der dritte vom Balaton. Der Fuss war hell grünlich-gelb.

Numenius ph. phaeopus L. — Nach den Hinweis Dr. Dorning's hatte A. Zergényi diese Art am 28. II. 1922 bei Keszthely beobachtet (Aquila 1922, p. 131).

Clidonias h. hybrida. Pall. — Am 12. IX. ein Stück im Ruhekleid am Strande von Balatonzamárdi zwischen 3 Flussseschwalben. Das erste Vorkommen am Balaton.

Larus minutus Pall. — Am 18. IX. zeigte sich ein Paar weit draussen am freien See vor Siófok.

LITERATUR.

Gaal, G. v. (1897). Orn. Jahrbuch. **8.** 10.
Gaal, G. v. (1901). Aquila. **8.** 295.
Gaal, G. v. (1902). Orn. Jahrbuch. **9.** 30.
Gaal, G. v. (1903). Aquila. **10.** 215.
Gaal, G. v. (1903). Balatoni Múzeum Egyesület Évk. **1.** 43.
Kleiner, A. & Vasvári, M. (1942). Magy. Biol. Kut. Munk. **14.** 132.
Keve (Kleiner) A. & I. Pátkai; & A. Vertse; (1943), Magy. Biol. Kut. Munk. **15.** 154.
Pátkai, I. (1942). Magy. Biol. Kut. Munk. **14.** 232.

METHODS OF BIRD SOCIOLOGICAL SURVEY, ON THE BASIS OF SOME TIHANY COMMUNITIES INVESTIGATED.

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The task of bird sociological research leads, in the last resort, in two directions. The one is sociological zoo-geography: We must describe bird populations of the biocoenosis living in the different regions of the world and the regularity in their existence, origin, extent; and changes which occur in these in place and time. The other general aim of investigations of this nature was formulated by PALMGREN (1930). He considers the production-biology of natural communities the most important synecological task. But the direction of production-biology in terrestrial synecology has not developed nor enlarged sufficiently in the past 15 years to advance investigations in bird ecology very much or to attempt to systematize the relations of bird populations in a metabolic system in some sort of biotic community.

Until now, however, while attaining one level with ecologists of other animal groups and being able to unite with them in common work, two fundamental tasks have remained to the ornithologist.

The one: population census work. We must express through statistical data the bird populations of the different community types, whether by individual count or with the aid of weight-quantities, more important from the standpoint of production-biology; and we must clear up the relation of these numbers to the changes due to ecological factors (PALMGREN, 1930, p. 79—80).

The other: On the basis of the necessary population surveys, we must describe the bird sociations existing in the communities of the larger districts investigated, and their characteristic qualitative and quantitative composition, together with data on their geographical extent.

Investigations of this nature have scarcely been undertaken in

Central Europe (SCHIERMANN, 1930. 34, 39. WÜST, 1931, STEINBACHER, 1942. SRÁMEK-HUSEK 1944.). The material I gathered during the war in the open woodlands of the Hungarian plain was unfortunately destroyed before publication.

In my present working territory, the Tihany Peninsula of Lake Balaton, ornithological research has been going on since 1938. In 1941 the Royal Hungarian Ornithological Institute established a permanent observation service on the peninsula which, in the later difficult years, limiting itself to the most important seasons, held out until the autumn of 1944 (KEVE, PÁTKAI, VERTSE, 1942, 1943, KEVE, PÁTKAI, UDVARDY, VERTSE, 1947,). PÁTKAI (1942) supplied useful assistance in many respects by his estimation of the 1941 breeding population which, without ecological or sociological evaluation, was published simply as raw census material.*

RELATIONSHIP OF THE ECOLOGICAL AND FAUNISTIC CONDITIONS DURING THE PERIOD INVESTIGATED.

The peninsula is one of the oldest parts of the Balaton district to submit to cultural influences. Since the middle of the 11th century it has constantly been inhabited and it can be assumed that cultivation of the fields, later of the vineyards, continued uninterruptedly on the peninsula's inner plains. In consequence of the economic methods employed the fields which submitted to grain and hoe-crops, the pastures and fallow ground were constantly interchanging, the orchards and vineyards increasing gradually in extent. On the uncultivatable slopes the forests also died out in consequence of pasturing. In the last half century forestry has been carried on upon these slopes and on the summits of the hills, so that the forests of today are, except for some smaller spots, relatively young and, because of the quality and lie of the land, as well as the climate, they reach no great heights.

Physiographically the peninsula consists of a basin cut in two and surrounded by wooded hills. In the smaller part is the "Inner Lake"; the "Outer Lake" is today replaced by a marsh-like meadow. The hill-tops are on an average 100 m higher than the surface of the water of Lake Balaton (the highest point is 232 m above sea level). The lakeside slopes are steep, with rifts and rocky walls and dense, bushy forests.

* In what follows the sociological terminology is after PALMGREN (1930), the avifaunistical after SALOMONSEN (1946). The "population" (sociation auct.) denotes the animal members of a systematic group of an association. Biochorion = habitat auct., biocoenosis = biotic community (biome S HELFORD'S), its unit is the association.

On the inner slopes the remains of dying forests, gardens and pastures mingle with cultivated areas. The cultivated units make an intricately detailed mosaic.

Of 12 sq. km. on the peninsula, except for the more or less adjacent grain fields, the greatest single unit is the Outer Lake meadow, about 70 ha, about 650x1000 m in greatest length and breadth, the greatest scrubby pasture is the Szarkád balk, about 65 ha, roughly 800x1100 m. The largest adjacent forest areas are about 20 and 14 ha, or 550x300 m and 800x350 m in extent. The largest orchard (lavender plantation with almond trees) about 26 ha, 800x300 m; the largest vineyard about 6 ha, 300x150 m; reedy pond 30 ha, about 800x600 m.

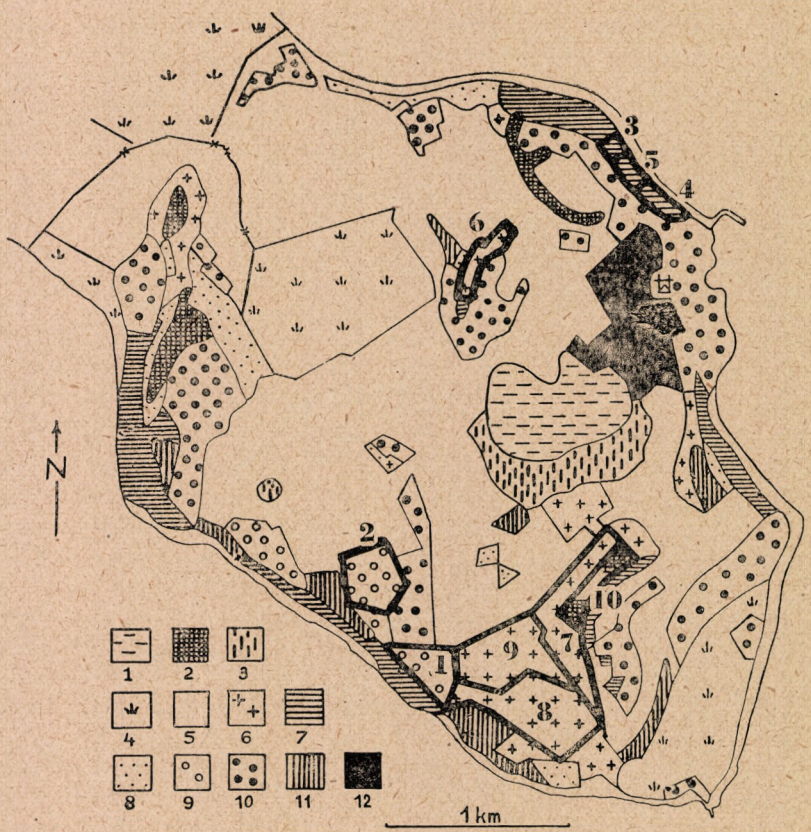


Figure 1. — Biochoria of the Tihany Peninsula from the standpoint of bird ecology, with the stations in the 1946 quantitative surveys. 1. Lake. 2. Grassy hilltops. 3. Pasture. 4. Meadow, marshy meadow. 5. Arable land. 6. Scrubby pasture. 7. Closed forest. 8. Shrubbery. 9. Open woodland. 10. Gardens, vineyards, orchards. 11. Cultivated forest. 12. Tihany village.

TABLE I.

Grouping of landscape elements of the Tihany Peninsula, from the standpoint of breeding bird populations.

Biochorion-types:		Plant association groups:	Characteristic association:
1	Reedy pond.	Phragmition	Scirpeto-Phragmitetum (Consoc. Typhetum)
2	Rocky- and loess cliffs, bare rocks, larger quarries.	Without macrovegetation	
3	Rocky tops covered with grassy vegetation.	Festucion sulcatae	Festucetum sulcatae (stipetosum) Festucetum pseudovinae
4	Pastures.	Lolion perennis Festucion sulcatae	Lolieta Festucetum pseudovinae
5	Meadows, marshes.	Agrostidion albae Magnocaricion elatae	Agrostideta
6	Arables.	Secalinion	
7	Ruderalia.	Lolion, Poligono-Chenopodion, Onopordion, Hordeion murini	
8	Scrubby pastures.	Complexes of Lolion-Festucion sulcatae-Prunion spinosae	
9	Gardens, vineyards, orchards.		
10	Shrubberies.	Orneto-Ostryon (and complexes with Festucion sulcatae	Querceto-cotinetum
11	Open woodlands.	Quercion pubescentis-sessiliflorae	Quercetum stepposum
12	Closed forests.	Fraxino-Carpinion	Acereto-Fraxineto-Ulmetum.
13	Cultivated forests, hedges, groves, groups of trees.		Robinieta, Pineta Pinetum cultum juv. etc.
14	Houses, buildings, and other structures.		

The areas heavily outlined on the map and numbered 1—10 are the stations of the quantitative survey (See Table III.)

I group the principal landscape types which serve as habitat to the biotic communities from the standpoint of the breeding birds under Table I. which follows. If they are covered with vegetation I mention the plant associations or their systematic category. The distribution of the biochorion types is to be seen on the map of the peninsula (Figure 1).*

Table II. shows the distribution of the breeding birds of the peninsula in the breeding season according to separate biochorion types. Those which did not breed during the year of my investigations are shown in parenthesis.

The faunistic picture of the area in the period of my investigations, in the breeding season of 1946, should be completed with those sorts which then resided there as regular summer visitors but which did not nest on the peninsula but in the neighbouring districts:

Coccothraustes coccothraustes L.
Apus a. apus L.
Falco p. peregrinus TUNST.
„ *ch. cherrug* GRAY.
Buteo b. buteo L.
Accipiter g. gentilis L.
Milvus m. migrans BODD.
Ardea c. cinerea L.
Anser anser L.

I must remark that the 5 sorts of birds of prey probably nest in the mountainous woods of the immediate neighbourhood in the Balaton uplands, the peninsula belonging to their normal feeding ground.

Also nesting in the closest vicinity but occurring on the peninsula only during migration or during their autumn and winter dispersion are:

Serinus canarius serinus L.
Lululla a. arborea L.
Certhia f. familiaris L.
„ *b. brachydactyla* BREHM.
Parus c. caeruleus L.
Phylloscopus c. collybita VIEILL.
Phylloscopus trochilus fitis BECHST.
„ *s. sibilatrix* BECHST.
Locustella fluviatilis WOLF.
Monticola saxatilis L.
Dryobates m. medius L.
Falco v. vespertinus L.

Breeding during the last 10 years but not observed to breed in 1946:

* On the basis of Professor SOÓ'S (1931, 32) works on the vegetation of the peninsula and of subsequent changes due to cultivation, personally conveyed by L. FELFÖLDY.

TABLE II.

The distribution of breeding birds according to biochorion-types.
( ) = In parenthesis those which did not breed in 1946. Nomenclature according to HARTERT (1903—22).

	1. Reedy pond.	2. Rocky and loess cliffs.	3. Grassy hilltops.	4. Pastures.	5. Marshy meadows.	6. Arable land.	7. Ruderalia.	8. Scrubby pastures.	9. Gardens, vineyards.	10. Shrubberies.	11. Open woodlands.	12. Closed forests.	13. Cultivated forests.	14. Houses, structures.
Corvus corone cornix L.											+	+	+	
Coloeus monedula turrium BREHM		+									+			
Pica p. pica L.												+	+	
Garrulus g. glandarius L.												+		
Sturnus v. vulgaris L.											+			
Oriolus o. oriolus L.									+	+	+	+	+	
Chloris c. chloris L.									+	+	+	+	+	
Carduelis c. carduelis L.									+			+	+	
Carduelis cannabina cannabina L.								+	+				+	
Fringilla c. coelebs L.									+	+	+	+	+	
Passer d. domesticus L.														+
Passer m. montanus L.									+		+		+	+
Emberiza c. calandra L.					+	+								
Emberiza c. citrinella L.								+		+	+	+		
Emberiza schoeniclus stresemanni STEINB.	+													
Galerida c. cristata L.							+							
Alauda a. arvensis L.			+	+	+	+								
Anthus c. campestris L.			+											
Anthus t. trivialis L.											+			
Motacilla f. flava L.					+									
Motacilla a. alba L.		+												+
Sitta europaea caesia WOLF.											+			
Parus m. maior L.									+	+	+	+	+	+
Parus palustris communis BALDENST.											+	+		
Aegithalos c. caudatus L. & Aegithalos caudatus europaeus HERM.									+			+	+	
Lanius minor GM.											+	+	+	

	1. Reedy pond.	2. Rocky and loess cliffs.	3. Grassy hilltops.	4. Pastures.	5. Marshy meadows.	6. Arable land.	7. Ruderalia.	8. Scrubby pastures.	9. Gardens, vineyards.	10. Shrubberies.	11. Open woodlands.	12. Closed forests.	13. Cultivated forests.	14. Houses, structures.
Lanius c. collurio L.								+		+	+	+	+	
Muscicapa s. striata PALL.									+		+	+		
Lusciniola m. melanopogon TEMM.	+													
Locustella l. luscinioides SAVI.	+													
Acrocephalus a. arundinaceus L.	+													
Acrocephalus s. scirpaceus HERM.	+													
Acrocephalus schoenobaenus L.	+													
Hippolais i. icterina VIEILL.									+			+		
Sylvia n. nisoria BECHST.								+		+			+	
Sylvia b. borin BODD.										+				
Sylvia a. atricapilla L.											+	+		
Sylvia c. communis LATH.								+	+	+		+	+	
Sylvia c. curruca L.								+	+	+		+	+	
Turdus ericetorum philomelos BREHM.												+		
Turdus m. merula L.											+	+		
Oenanthe o. oenanthe L.		+					+	+						
Saxicola r. rubetra L.					+			+						
Saxicola torquata rubicola L.								+		+				
(Phoenicurus p. phoenicurus L.)									+					
Phoenicurus ochruros gibraltariensis GM.									+					+
Luscinia m. megarhyncha BREHM								+		+	+	+		
Erithacus r. rubecula L.											+	+		
Troglodytes t. troglodytes L.												+		
Hirundo r. rustica L.														+
Delichon u. urbica L.														+
(Riparia r. riparia L.)		+												
Caprimulgus europaeus meridionalis HART.												+		
(Merops apiaster L.)		+												
Upupa e. epops L.		+						+	+	+	+	+		
Coracias g. garrulus L.											+			
(Alcedo atthis ispida L.)		+												
Picus v. viridis L.											+			

	1. Reedy pond.	2. Rocky and loess cliffs	3. Grassy hilltops.	4. Pastures.	5. Marshy meadows.	6. Arable land.	7. Ruderalia.	8. Scrubby pastures.	9. Gardens, vineyards.	10. Shrubberies.	11. Open woodlands.	12. Closed forests.	13. Cultivated forests.	14. Houses, structures.
Dryobates maior pinetorum BREHM.											+			
Jynx t. torquilla L.									+		+	+		
Cuculus c. canorus L.								+		+	+	+		
Otus s. scops L.												+		
Asio o. otus L.												+		
Athene n. noctua SCOP.											+	+		
Strix a. aluco L.												+		
Falco s. subbuteo L.											+			
Falco t. tinnunculus L.		+												
Circus ae. aeruginosus L.	+													
Accipiter n. nisus L.												+		
(Ciconia c. ciconia L.)														+
Ixobrychus m. minutus L.	+													
Anas platyrhyncha L.	+				+									
Anas querquerdula L.	+													
Anas strepera L.	+													
Nyroca f. ferina L.	+													
Podiceps c. cristatus L.	+													
Podiceps g. griseigena BODD.	+													
Podiceps n.nigricollis BREHM.	+													
Columba oe. oenas L.											+			
Columba p. palumbus L.												+		
Streptopelia t. turtur L.										+	+	+	+	
Vanellus vanellus L.					+									
Tringa t. totanus L.					+									
Capella g .gallinago L.					+									
(Larus r. ridibundus L.)	+													
Rallus a. aquaticus L.					+									
Porzana porzana L.					+									
Crex crex L.					+									
Gallinula c. chloropus L.	+													
Fulica a. atra L.	+													
Perdix p. perdix L.						+		+						
Coturnix c. coturnix L.					+	+								
Phasianus sp.										+			+	

Emberiza hortulana L.	1936*	*Alcedo atthis ispida* L.	1936*
Phoenicurus ph. phoenicurus L.	1941**	*Ciconia c. ciconia* L.	1942**
Merops apiaster L.	1943***		

On the other hand, towards 1941** there were new breeding species. Indicating the minimum number of pairs which, according to my estimation, bred on the peninsula:

Anthus trivialis	2	*Caprimulgus europaeus*	1
Sitta europaea	1—2	*Picus viridis*	8
Aegithalos caudatus	8	*Asio otus*	1
Muscicapa striata	1	*Columba oenas*	2
Sylvia borin	7	*Tringa totanus*	1
Turdus ericetorum	2	*Perdix perdix*	10—12
„ *merula*	30—40		

Those which towards 1941 increased in numbers (compared with PÁTKAI's data):

	1941	1946		1941	1946
Sturnus vulgaris	1	8—10	*Parus palustris comm.*	1	2—3
Galerida cristata	3	6—8	*Erithacus rubecula*	1	4
Anthus campestris	1	2			

In what follows I do not concern myself with the water and cultivated biochoria. Rifts on the tops and slopes of rocky and loess cliffs looking on the Balaton are so numerous that the *kestrel* uses them exclusively as nesting places, whereas in other hilly regions it uses the nests of the magpie and crows which it also finds there plentifully. *Jackdaws* nest here also in colonies of 30—40 pairs, scattering for food in all biochoria with the exception of closely settled human habitations. The pastures and arable fields are poor in kind and quantity, in consequence of being divided up in small areas.

In the more natural biochoria we can establish the following order on the basis of pedological and microclimatological relations, descending from optimum to pessimum: open woodland; closed forests; shrubberies; scrubby pastures; grassy, rocky hilltops. There are on the peninsula 2 smaller individuals in the way of open woodland; among the old oaks here (about 80 years old) are many sorts of birds which are hole-breeders. 41.5% of the species here found are hole-breeders, while in the closed forest only 20%, but the latter are also more euryoecic species, content with temporary quarters. The closed forest is extremely varied, with a mixed forest growth: *Fraxinus ornus, Acer campestre* and *platanoides, Ulmus glabra* and *scabra, Quercus sessilis* and

*HOMONNAY, 1938, l. c.

**PÁTKAI, 1942, l. c.

***KEVE-PÁTKAI-UDVARDY-VERTSE 1947, l. c.

lanuginosa, Carpinus betulus, Pirus piraster, Cornus mas. Among these are planted *Pinus silvestris* and *austriaca, Robinia, Ailanthus,* which are sometimes found in quite separate groves (13. biochoria). In the shrubberies are the *Quercus lanuginosa, Cotinus coggygria, Fraxinus ornus, Crataegus monogyna, Prunus spinosa, Cornus sanguinea,* etc. The last degree of destruction of the forests, in consequence of pasturing, is the scrubby, thorn-bushed pasture *(Crataegus monogyna, Prunus spinosa, Rosa sp.* etc.). On the driest, warmest, sunniest places, the hilltops generally, the shrubbery gives way to grass. These biochoria make small individual mosaics on the peninsula which often interchange with one another or overlap.

In regard to breeding birds these 5 biochoria can be established in similar order, with the difference that in respect to qualitative and quantitative distribution the closed timber forests stand out foremost. I have not yet sufficient data to present the quantitative relations; the qualitative distribution and the relation between the 5 biochoria can be seen in Figure 2. The change in order is explained in its quantitative aspect on the Figure by the physiognomic profile of the biochoria: the plant community of most strata and of the most varied life form structure is the closed forest. The order is changed by the relative distribution of the species, because of the numbers of stenoecic species of the closed forest, in respect to which it must be remarked, however, that the open forests, as has been stated, consist of few and small individuals. Therefore of the characteristic sorts* only the largest „K" values could be considered in my investigations.

On Figure 2. I have distinguished the hole-breeders because of their special ecological claims.

The physiognomic profile seen from the viewpoint of bird ecology explains again the relation between the 5 biochoria and the placing on the Figure of the bird species belonging thereto. The hole-breeders live in the 2 forest communities, proportionally to the development of the trunk strata in the forest.** (Groups L — 0). From this exclusive mass stand out the 2 most stenoecical species, the *Parus maior* and the *Upupa epops,* which go so far as to find nesting places in root holes and rifts in rocks. The other group of forest species extends together with the completely developed high tree-canopy, that is, with the real forest (Group A). Group B is found really everywhere where we find

* Characteristic sort = highest degree of fidelity (F.)

** Felföldy 1943.

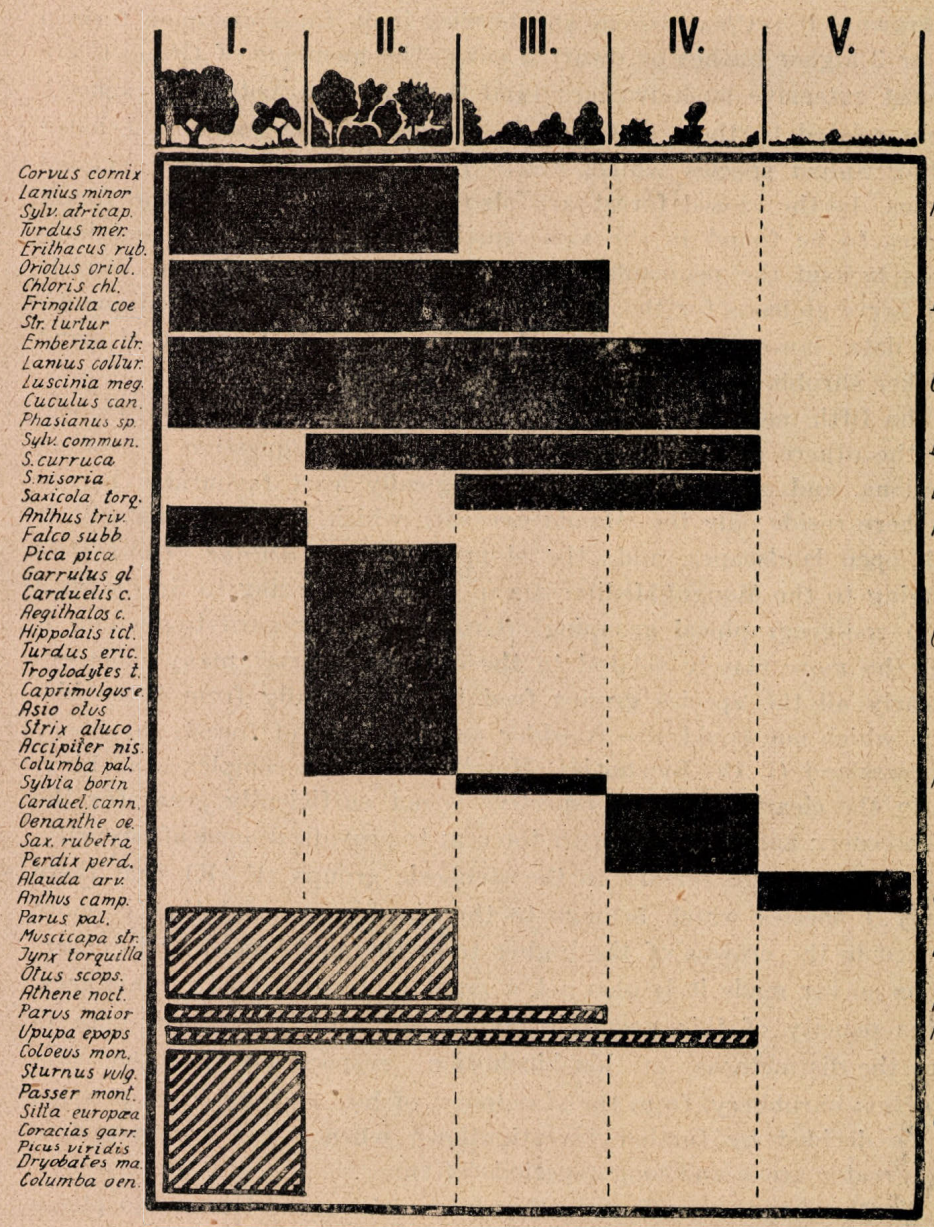


Figure 2. — The bird ecological relation in the biochoria resulting from the half-wild plant communities, based on the qualitative investigations of breeding fauna in 1946 (and partly combined with PÁTKAI's 1941 data). A—E = species groups which extended over several biochoria (euryoecic species). F—K = species groups which live in only 1 biochorion (stenoecic species). L—O = hole-breeders in similar groupings.

tree crowns. It settles also in shrubberies if it finds canopies formed by 2 or 3 higher bushes or dwarfed oaks. The members of Group C are the most extensive bush-dwellers (and their nest-parasite, the *cuckoo).* Closely related to them is Group D, formed of the commonest bush-dwelling *Sylvia* species which, however, do not live under closed canopies. In the closed forest are also the inhabitants of the forest margins, to be found in the same quality in the open woodland individuals beyond the peninsula. They are lacking only in the records which serve as basis for the present Figure. The not-indicated quantitative data, however, show that those belonging to Group C demand a thicker shrubbery, especially the *Luscinia megarhyncha.*

The fifth, merely grass-covered biochorion differs in every respect from the others on the Figure. Here too it is a question of nesting conditions, and on the mountains' ecologically least favourable barren tops there reside only the *Alauda arvensis,* which with us adapts itself to all open landscapes, and the steppe-dwelling *Anthus campestris* belonging to the Mongol-Mediterranean group, according to STEGMANN's (1938) zoo-geographical grouping. (Group K.) In regard to feeding it is all the more closely related to the other biochoria: many members of nearly all the species groups feed here, especially from those biochoria which one could also consider as complexes of forest, shrubbery and grassland (I, IV. Biochoria). However in these complexes no birds nest in the clearings; the perspective is not sufficiently great for the grassbreeders, and the thickets are too near to providie a sense of security.

It is worthy of remark in the stenoecic groups (F—K) that there is only 1 species which belongs to the shrubbery, and that is more attached to the crowns of the higher trees therein. According to observations so far made it is also not a regular breeding bird in this area. So the paucity of this group in comparison with the others puts in doubt the distinctness of this biochorion, respectively the *Querceto-Cotinetum* shrubbery, from the standpoint of bird ecology. This question will be decided by further investigations, when I can compare data from greater areas and longer periods of time.

Weather conditions. The weather in general in the Hungarian plains falls between the oceanic and the continental climate, the climate of the Balaton district being characterised also by dryness, with wide extremities in temperature (RÉTHLY, 1940). In the first half of 1946 there was warmer and drier weather than usual. Below I give the principal data, comparing the average and mean values over a period

of 10 years (from the data of the Meteorological Station of the Hungarian Biological Research Institute, contributed partly by BACSÓ, l. c.).

	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1931—40
Mean yearly temperature °C	10.3	10.7	10.0	12.4	11.0	11.5	11.6	11.4	11.5	8.9	10.9
Annual mm rainfall	606	410	590	539	462	762	795	434	600	832	603
			I.		II.	III.	IV.		V.	VI.	I—VI.
Monthly mean temperature °C	1931—40		—1.0		0.6	5.5	10.1		16.5	20.1	5.2
	1946		—2.8		3.4	6.9	14.1		18.8	21.5	6.2
Monthly rainfall mm	1931—40		31		39	38	32		77	59	276
	1946		20.3		40.6	31.3	1.9		72.4	69.6	236

As we see, April, 1946, was remarkably dry and was also relatively the warmest month.

THE SOCIOLOGICAL SURVEY.

Among previous bird census methods only those based on a square unit could considered, as my aim was to achieve absolute values. Because of its sociologic usefulness I used the quadrat method. In most cases I did not work in a regular, exactly measured square area, but in irregulary formed areas bounded by topographical features or by the limits of the biocoenosis itself, whose dimensions were easy to read off, or to reckon, from the land register or from the numerous large-scale maps at my disposal (maps at the scale of 1:5000, 1:20,000, 1:25,000, etc.).

I made quantitative investigations in 10 stations, in all on 28 occasions, the first on April 9th, 1946, gathering information, the others between April 26th and June 12th. The surveys were made from sunrise, or, according to Middle European time, from about 4 to 8—9 a. m. In May and June, because of the great heat, complete surveys cannot be made after 9 or 10 o'clock. No surveys were made during rainy or very windy weather. The greatest extent covered during one survey was about 17 ha in the scrubby pastures, about 9 ha in the open woodland, and about 2 ha in the closed forest.

I found that in our circumstances a larger area could not be controlled on one occasion. The net system observation hypothesis was modified by topographical conditions and in reality could be carried

out completely only in the open woodland. In the scrubby pastures where the bushes rose above eye-level I counted always only in one direction, towards the area already counted, in order not to inscribe the same bush twice in the midst of wandering among the thorn bushes. In dense undergrowth of the closed forest I guided myself by compass.

The open woodland (1st and 2nd stations) provided the easiest work; the oak trees standing apart from one another not making a really closed canopy and 12 to 15 m high could be seen through easily. The clearings among them were in general composed of low *Festucetum pseudovinae* turf with few and scattered bushes. On the other hand, the closed forest (3. 4. 5. stations) in places covered steep and gullied hillsides, with closed canopy stratum 15—20 m in height, under or between which were often the closed crowns of young trees 6—10 m in height. Here and there the undergrowth is scarcer and only 2—3 m high. In other places the blackberried elder especially makes a canopy closed together with the tops of the trees, under which the vegetation of the herbaceous stratum is often lacking, while on the contrary there is an entanglement of decayed leaves, rocks and dried shoots. In these places the counting is the most difficult. Here continuosus advance in a given direction is impossible. In all such small thickets it took 5--10 minutes until I could identify the 8—10 singing birds which all at once appeared in my sphere of observation, and could establish which were the examples which had accompanied me from neighbouring, already examined, areas by hearing their notes of alarm or song; these after a few minutes returned to their own territories. Thus especially on one occasion 5—6 males of the chaffinch *(Fringilla coelebs)* accompanied me. If I stood still and ceased moving the colour of my clothing merged with that of my surroundings, their feeling of danger diminished, and they immediately began to quarrel with one another, making their control still more difficult. It soon developed in the course of my experiences that here one cannot, as PALMGREN recommends, work with a previously prepared notebook in which one must merely make a stroke after the name of the species. This makes easier the working up of a still larger territory. Here we must choose a small area and therein also distinguish small, detailed parts. In so thick a wood we must repeat the survey more than three times rather than less. On the second or third occasion the topographical features are not so troubling and the orientation is easier. And if the data of the notebook are prepared in the order of what is seen and this is in the hand during the control it makes the later surveys less difficult. On the

other hand, the mapping of the nests or territories (VÄLIKANGAS, 1937) in such a dense bird population is a much greater undertaking. I call attention to the fact that in a similar physiognomic but level *Quercetum convallarietosum* (the forest of Ohat on the Hortobágy steppe, in the summer of 1943) the surveys involved just such difficulties.

The 6th station was a mountain slope bounded by a rocky ridge and, in one small part, cultivated spots and vineyards, with closed shrubberies and forests in the parts falling outside the area of my station. The station investigated stands physiognomically closer, partly to the scrubby pasture and partly to the closed shrubbery. It was not regularly used for grazing. The turf of the clearing consists of *Festucetum sulcatae stipetosum;* its bush groups are more united, most of them higher than 1.5 m, and the thorny species have a more subordinate role.

The 7th, 8th and 9th stations abut on one another. Cattle graze in these shrubberies in summer, in spring and autumn the swine. The surface is irregular with heights of 30—50 m which are full of limestone rocks 3—5 m high (cones of ancient geysers.) The tuff-rocks are here and there hollowed out and *Oenanthe oenanthe* and *Upupa epops* use them for nesting. On about a third of all three territories the bushes are lowered to about 1.20 m, that is, chewed away, with clearings which extend to 60—100 m in diameter. The other part has clearings at most 40 m in diameter with groups of bushes about 2 m in height, most of them *Crataegus monogyna* and *Rosa sp.* Arable fields bound a third of the scrubby pastures, forests a further half, while the rest is vineyards. Of the third of the 8th and 9th stations bounded by open woodland the shrubbery is almost entirely cleared, indeed remains of former forests are to be found there with groups of 2—3 oak trees, the highest reaching 8 m. These peculiarities and the effect of the neighbouring biochoria can be demonstrated in the qualitative composition too by the presence of the accessory and accidental elements. In the part bounded by the closed forest and the *Pinetum cultum Chloris, Fringilla,* and *Turdus merula* are numerous and in the neighbourhood of the open woodland the *Oriolus, Falco subbuteo* and *Pica* were often to be seen. The 10th station belongs to the same shrubbery but is surrounded by stone walls and thus is not used for pasture. In it is a long ridge with plantations of *Pinus austriaca* in places 1—1.20 m in height and poor in birds, with single *Ailanthus glandulosa* shoot-growths, remains of shrubbery not higher than 1 m and, in general, barren rubble or rock surfaces richly covered with grass. The two

grass-inhabiting sorts, the *Alauda* and the *Anthus* can breed in security here in the protection of the stone walls and for that reason do not breed in the similar neighbouring bare grassy tops of the 7th station.

For all stations except the 3—5th closed-forest stations I give 2 columns of figures. In Column A are the total original data, including all the individuals of all species seen during the surveys. In Column B only those individuals which in that time nested there. I shall return to this later.

From the known sources of error of the quantitative surveys (PALMGREN, 1930, I. VÄLIKANGAS, O. KALELA, J. SOVERI, l. c.) there are eliminated as far as possible those arising from measurements of the territory, the weather, the disposition and experience of the observer. I determined whether some doubtful individuals seen at the boundaries of a station belonged therein or not on the basis of whether the center, or at least the greater part, of their breeding territory lay within the station or not. In characterizing the wooded stations I have already spoken of the too great forest and bird density of which Eastern Europe is capable and which are sources of error obliging departures from habitual methods.

To the question whether in the breeding period we count the pair or the individual, I have two observations to make: The one, that on the 14th of May in the 7th station on an area of 2 ha I found 6 male *Lanius collurio* among which were 3 which behaved as if they belonged there. On the 28th of May in the same place I found 2 nesting pairs. In the first half of May the migratory-passage of the *Lanius collurio* was still going on over the whole peninsula, thus certainly 3 passage visitor males belonged to the quantitative survey made on the 14th of May, indeed, as I counted in pairs, 3 pairs. The other case, to which I shall refer again, was that in the same place, also on the 14th of May, there was present a flock of about 150—160 *Coloeus* which were not to be seen afterwards. These too are given in pairs wherever indicated.

To control the suitability of the quadrate method I made an oft-repeated count in 3 stations of the closed forest. In spite of the fact that these repeated surveys lasted over a long period, during which qualitative and quantitative changes could certainly have taken place in that area I carried out this control in respect to such numbers of pairs, that is, the suitability of counting a given abundance of birds thus.

The evaluation was carried out entirely after PALMGREN's statistical method (1930, p. 84—91). I reckoned the increase in abundance-values occasioned by the repeated counting from the register of each station; that is, by what percent the total is increased due to divergences between I and the total and II and the total*; by what percent the abundance increases in the I + II + III total in comparison with the I + II, I + III, II + III; and so on. Combining all the possible cases I got the relation between the results of the single cases counted (I + II, II + III, III + IV, and so on) as a percentage of the smaller values. These percentages I ranged in 5% divisions. I reckoned their median. (In the 3 stations from the results of 17 surveys I got between the 1 and II counts 84% values, between the II and III 177, between the II and IV 204, IV and V 135, V and VI 48. Between the VI and VII I got only 6 values, therefore I did not concern myself further with this one.) Expressing these media on a logarithmic scale I got the following value-range:

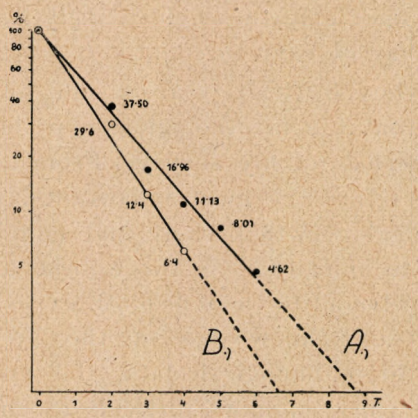


Figure 3. — The percentual increase of bird density count made by repeated surveys. Abscissa (T) = number of surveys made. Ordinate (%) = the graphing of the percentual increase of bird density counts made in comparison with the results of previous surveys, drawn with the median of the cases on simple logarithmic scale. Line A = the values obtained from the surveys of the closed forests of Tihany (Stations 3, 4, 5). Line B = the graphing of the values counted from PALMGREN's control material (1931, l. c. p. 84—91) which he got from surveys made in the forests of Åland.

* As the result obtained is a minimal number, the sum of 2 or more countings, it was so made that in all species the greatest number found was calculated PALMGREN, 1930, p. 84).

In comparing my results with PALMGREN's, the discrepancy is not so great as to prohibit the use of his method. I abstained from further use of this reckoning and the use of a correction. In the case of the present research this attitude towards the correction is specially justified (VÄLIKANGAS, 1937, p. 49 SOVERI, 1940 p. 13—14) insofar as it gives an incorrect picture of the qualitative distribution and among other things it changes the values of the individual dominance which I had also reckoned. It appears from my surveys that the qualitative composition from station to station is diverse enough within the same community; but my records were insufficient to express with sociologically relative figures the small value of the homogeneity.

Reckoning briefly the results of the percentual differences between the I and I + II estimated result got from the 3 surveys of the scrubby pasture, the average of the 6 values is 22.25%. In the same case the median according to PALMGREN is 29.5%, in my results in closed forest 37.5%. This reckoning confirms my experience that the scrubby pasture is easy to see through and in the period of the survey the species living therein lived a life predominatingly bound to their territory, loud and active; that is, easy to count.

In my researches difficulties arose not hitherto encountered in the literature, in that the ornithological aspects merged into one another during the periods of my surveys. From the standpoint of quantitative records therefore the most important is the population census made in the principal growth period (that is to say, the breeding period) because a.) technically it is the easiest to count at such times the pairs which are bound to their nests, i. e., to their territory; b.) we can establish from our knowledge of the breeding-bird population, with the help of brood-biological data, the probable number of the estival population which is more difficult to count. Moreover, in our zoogeographical units, except the steppes, the most characteristic biocoenetically is the avifaunistical category "nidiates" after SALAMONSEN (1946, p. 42.). To explain the difficulties mentioned I show below the ornithophaenological spectra of the closed forest community of Tihany (the area of stations 3.—5.) and the scrubby pasture (area of stations 7.—9.) in the period of my surveys in their vernal and pre-estival aspects (Figures 4 and 5).

53% of the nidiates of the closed forest are residents and begin their nesting in March, at least in its second half; or at least choose and occupy their territory. At this time a good many of the winter visitors are still here too. The greater part of the summer residents

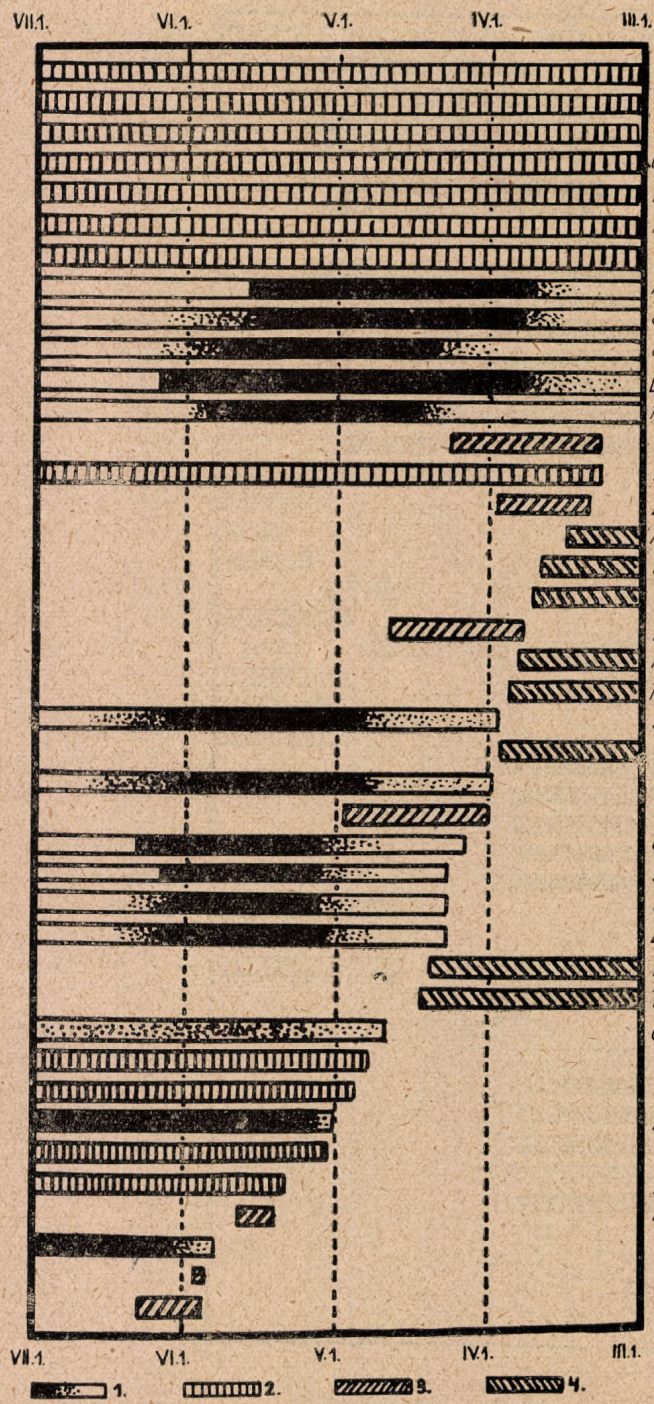
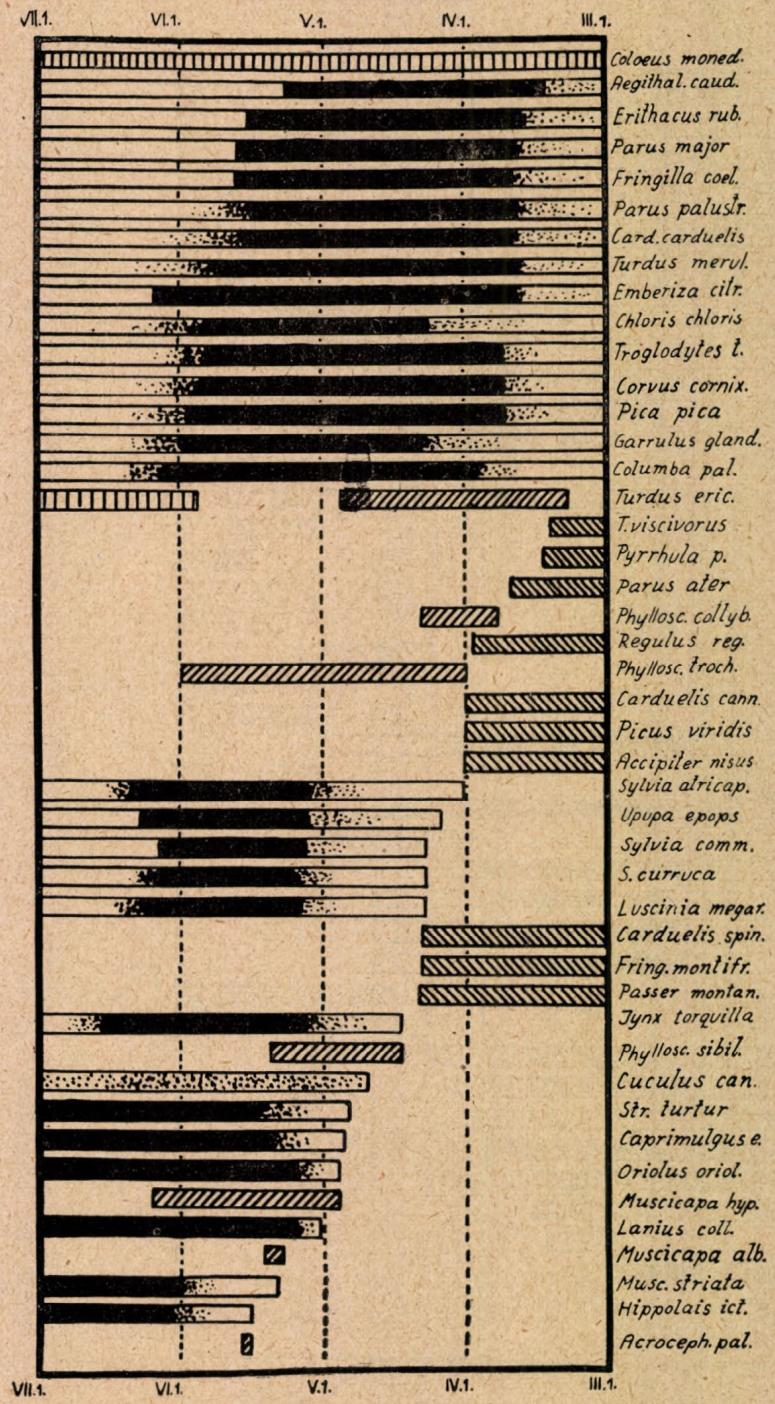


Figure 4. — The bird phaenological spectrum of the *Aceretо-Fraxineto-Ulmetum* (closed forest) of Mt. Óvár in Tihany between March and June, 1946. For explanation of symbols see Figure 5. p. 79.


VII.1.
VI.1.
V.1.
IV.1.
III.1.
Coloeus moned.
Aegithal. caud.
Erithacus rub.
Parus major
Fringilla coel.
Parus palustr.
Card. carduelis
Turdus merul.
Emberiza citr.
Chloris chloris
Troglodytes t.
Corvus cornix.
Pica pica
Garrulus gland.
Columba pal.
Turdus eric.
T. viscivorus
Pyrrhula p.
Parus ater
Phyllosc. collyb.
Regulus reg.
Phyllosc. troch.
Carduelis cann.
Picus viridis
Accipiter nisus
Sylvia atricap.
Upupa epops
Sylvia comm.
S. curruca
Luscinia megar.
Carduelis spin.
Fring. montifr.
Passer montan.
Jynx torquilla
Phyllosc. sibil.
Cuculus can.
Str. turtur
Caprimulgus e.
Oriolus oriol.
Muscicapa hyp.
Lanius coll.
Muscicapa alb.
Musc. striata
Hippolais ict.
Acroceph. pal.
VII.1.
VI.1.
V.1.
IV.1.
III.1.

(Fig. 5.)

arrive during April. Those arriving early in the month are already bound to their nesting territory by the latter half of the month. Passage visitors are relatively few; on the other hand most of those which pass over stop for a lengthy time in the community. As the forests come into leaf the last winter visitors disappear. In the first half of May the late-comers arrive and by the end of the month all have taken up their breeding territories. But looking at the beginning of the spectrum we see that the earliest breeders have already got their young out of the nests, which from the standpoint of quantitative records brings their localized habits to an end. If we had made quantitative records in March it would have been interesting only in respect to abundance of residents. The animal and plant life which begins after the snow melts has, according to my observations, no influence on the abundance of birds, because, as I saw, one can see neither new species nor an increase of the old ones to any great extent. Indeed, the diminution of the winter visitors must result in a reduction of the bird population. The optimal time of quantitative recording of the hibernating residents is April. But at that time the quantitative picture of the whole bird population changes from day to day as the migrators arrive one after the other; indeed from among the migrants there remain for a time with us groups or examples which do not breed in the area. (See the flocks of *turtle doves* flying through the scrubby pastures observed until the end of May and the already mentioned passager *red-backed shrike* males.) The first 3 records of the 4th station show that the bird population observed increased almost by 100% from the beginning of April to the beginning of May, and by 288% of the total result of the 3 surveys (calculated by the method described in the footnote on

Figure 5. — The bird phaenological spectrum of the *Pruneto-Crataegetum+Festucetum pseudovinae* (scrubby pasture) of the Szarkád balk. March-June, 1946. 1. Breeding species. The period when they are most closely attached to the territory (occupation, laying of eggs, hatching, care of young birds) is drawn in black; the time of slacker localization is shaded. A sharp limit is shown as end of the localized period in those cases when I knew the day the young left the nest. Among the early breeding sorts the first half of the second breeding cycle falls into this period (March-May), but this is not shown on the Figure because at that time the quantitative values are confused by the young birds flying about, thus as far as these species are concerned the principal spring quantitative investigation period is ended. 2. The species which normally live and feed on this area at this time but which do not breed there. 3. Passage visitors. 4. Winter visitors. Each species is graphed in a column of equal width whose beginning and end show the extent of its stay in the area.

page 77.); whereas the number of species by 88.9%. With the quantitative records for May we can reckon more exactly the population of the earlier arriving summer residents in the beginning of the month and the later arrivals at the end of the month. The *owls* do not occur in the spectrum, no more do they have a place in the quantitative surveys, partly because of their rarity in the territory and partly because they conceal themselves by day. I saw the *Asio otus* and the *Strix aluco* only in March and the *Athene noctua* and the *Otus scops* only in July.

The foregoing supports the evidence of the phaenological spectrum of the scrubby pasture. Here are few hibernating residents. That group, however, has a great role which in the closed forest is represented only by the *jackdaw;* the species not breeding in the community but feeding there, residents as well migrants. Otherwise in this spectrum there is not so remarkable a contrast between the breeding period of the residents and that of the migrants.

I find this mode of representation suitable for expressing total yearly qualitative and, combined with the individual dominance values, the quantitative changes of the bird population of a community; and therefrom the characteristics of the aspects of the bird population can be read.

Returning to the interplay of the limits of the vernal-preestival aspects on the basis of the spectra, I emphasize the fact that — referring to the other, not represented communities too — *that epoch when the localized period of most bird species coincided and was the most easily counted, lasted in our surroundings at the most for a week or 10 days. This caused a great difficulty in recording the population by the methods hitherto employed,* from which we must also gain sociological zoogeographical results besides the quantitative numerical data which are our production-biological aim. This difficulty — judged from the literature (PALMGREN, VÄLIKANGAS, SOVERI, KALELA, etc.) — does not arise in the research of the biotic communities of Northern Europe.

Another difference from northern conditions is the presence of non-breeding species, as can be seen from the scrubby pasture spectrum. These without doubt have another sociological role than that of nesting and feeding there. On the other hand, in those communities where they nest, they have also not the same value as the species

bound to the same communities in all phases of their lives. *So in a sociological characterizing of the bird population of a biotic community we must indicate with different marks whether the species is breeding or feeding, or both, locally — that is, in a separate, observed community — or in the entire biotic community surveyed,* and which phase of life, in which biocoenosis, has the greater significance in the life of the entire community.

Further investigations are necessary to clear up this sociological characteristicness. But until then I shall not use the term fidelity (exclusiveness of a species in an area of a community) nor the term character-species, because the species mentioned can nowhere be classified in the exclusive plant sociological ranks adapted by PALMGREN. Seeing that the presence of these species also influences the individual dominance values in the Table showing the quantitative surveys, I have expressed in the records where such species occurred, besides the column of the original abundance values (A) in a separate column (B) the quantitative data determined from the species nesting in the area; and thus in Columns A and B are expressed the dominance values pertaining thereto.

Similarly unexplained in the communities examined is the role of the larger birds of prey. According to my observations, in the 2 forest communities in the nesting season this role was very insignificant. The *Falco tinnunculus* which nests in the stone walls of the forest as well as the *Falco subbuteo* feed in the open biochoria. One pair of *Accipiter nisus* nested on the peninsula in a forest of the *Acereto-Fraxineto-Ulmetum* type, but the feeding territory of this pair was not included within my stations. Neither did the *Accipiter gentilis,* which bred not far from the peninsula, feed in these forests. On the other hand, I observed in all the other biochoria regularly, if not from day to day, the *Buteo, Accipiter gentilis, Milvus migrans,* breeding in the woods 3—4 km distant and sometimes also the *Falco peregrinus* and *Falco cherrug.* Therefore all biochoria of the peninsula which are not closed forest belong to their regular feeding territory and thus they belong to them in sociological characterizing; but their role cannot be expressed with the sociological data thus far employed. In these species with us the use of the belt transect survey method („Linientaxierung" PALMGREN's) is the right one. It is more exact for searching out the biochoria which provide a possibility for breeding and for the counting there of every single nest. The investigaton of the role of the birds of prey led me to the reflection that *the constancy term of plant*

sociology is also not to be used regardlessly in bird ecology. Because, for example, during the qualitative observations the *Buteo buteo* occurred almost without exception on the scrubby pastures, that is, it was present on all of them every day. But it fell within the quantitative surveys only once, on the 5th of June, 1946, in the area of station 9. If then I want to express its constancy on the basis of the records, this value would be very small, differing from its, in reality, truly great constancy. However these birds which live in a territory with a greater radius of activity can have quite as great a constancy as the small singing birds which are to be seen easily and at any time. *From the standpoint of the expression of constancy, therefore, the quantitative methods nowadays employed are incomplete. The same is true of the local constancy as the term is used by plant sociologists.* For example, in the closed forest individual examined, which extend over 15 ha, there nested 2 pair of *magpies.* These could be found at their daily activities in one part or another of the forest or in the neighbouring biochoria. So that no matter how many stations I had investigated in the forest the *magpie* would never have seemed locally constant as it never occurred in 80—100% of the investigations, though it is certain that it was present daily in every part of the forest. And if I consider the previous examples from the standpoint of local constancy it appears that in all the scrubby pastures of the Tihany Peninsula it is the same specimen of *buzzard* which is active. It is in each place locally constant but I cannot establish this from my records. *The same incompleteness in method makes it impossible to reckon the minimi-areal.* Neither can the frequency scale worked out by Soveri (1936, p. 17) nor the Lindsdale-Compton scale he mentioned be employed in our country, consisting of mosaics of small individuals of plant societies. Lindsdale's frequency-scale was based on the time unit (unfortunately I don't know more of it); it shows the right tendency inasmuch as the significance of the difficulties described above is always *that the individual members of a population pertain at different times to different parts of the area: thus one must employ the time factor in their sociological characterizing method, in contrast to that of the localized plants.*

THE RESULTS OF THE SOCIOLOGICAL INVESTIGATIONS.

In the tabulated summarizing of the quantitative surveys, are given the abundance and individual dominance of the species for all stations, as well as the population density count per km^2, by adding the abundance values of all species. In the biochoria from which I have more

records the average density of population was counted as well as the average values of individual dominance.

Per km² the density of bird population in nesting pairs is:

Open woodland	690
Closed forest	4056
Scrubby pasture	410

All pairs observed in the area:

Open woodland	750
Scrubby pasture	695

Comparing these with the results of other researches (LACK, 1937, PALMGREN 1930, 1931, 1933, 1938, SOVERI, 1940) the too high density values of the closed forest stand out. According to the authors just mentioned the greatest value so far found was, in Finland 719, in England 2000. My high figure can be explained by the geographical lay, the more advantageous climate of the area investigated, as well as the diverse strata and variability of the closed forest which with us is the climatic-climax community; indeed, in some of our woods ecologically even more favourable, the bird population is probably still denser..

The bird population of the 3 biochoria examined in most detail can be classified in the following categories, with the help of individual dominance groups (PALMGREN, 1930, p. 172,). Only the nidiates were taken into consideration.

THE BIRD POPULATION OF THE OPEN WOODLAND.

Dominance groups: > 5% dominant, 5—2% dominant, 5—2% influent, 2 > % accessoric.

Dominant	D
Fringilla coelebs	13.8
Streptopelia turtur	12.3
Emberiza citrinella	11.2
Sturnus vulgaris	7.4
Parus maior	7.3
Influent	D
Mucicapa striata	4.5
Oriolus oriolus	4.5
Corvus cornix	3.9
Sylvia nisoria	3.3
Luscinia megarchyncha	3.3
Turdus merula	2.8
Upupa epops	2.5
Sylvia atricapilla	2.3
Jynx torquilla	2.2
Lanius minor	2.2

Accessoric	D
Falco subbuteo	1.7
Passer montanus	1.6
Anthus trivialis	1.2
Cuculus canorus	1.2
Coloeus monedula	0.6
Dryobates maior	0.6
Coracias garrulus	0.6
Sitta europaea	0.6
Picus viridis	0.6
Columba oenas	0.6
Erithacus rubecula	0.6

THE POPULATION OF THE CLOSED FOREST.

Dominant	D	*Accessoric*	D
Streptopelia turtur	9.9	*Corvus cornix*	1.9
Fringilla coelebs	9.2	*Sylvia curruca*	1.8
Emberiza citrinella	8.8	*Cuculus canorus*	1.8
Luscinia megarchyncha	8.3	*Garrulus glandarius*	1.5
Parus maior	7.6	*Aegithalos caudatus*	1.5
Sylvia atricapilla	5.8	*Parus palustris*	1.3
Turdus merula	5.8	*Sylvia communis*	0.7
Oriolus oriolus	5.3	*Jynx torquilla*	0.7
Lanius collurio	5.0	*Muscicapa striata*	0.7
		Upupa epops	0.7
Influent	D	*Hippolais icterina*	0.7
		Pica pica	0.6
Chloris chloris	3.8	*Erithacus rubecula*	0.6
Carduelis carduelis	3.3	*Troglodytes troglodytes*	0.6
Columba palumbus	2.0		

THE POPULATION OF THE SCRUBBY PASTURE.

Dominant	D	*Accessoric*	D
Sylvia communis	24.8	*Fringilla coelebs*	1.5
Emberiza citrinella	21.0	*Alauda arvensis*	1.2
Saxicola torquata	10.9	*Streptopelia turtur*	0.9
Luscinia megarhyncha	9.7	*Cuculus canorus*	0.6
Carduelis cannabina	7.4	*Perdix perdix*	0.6
Oenanthe oenanthe	5.0	*Anthus campestris*	0.6
Influent	D		
Lanius collurio	3.6		
Saxicola rubetra	3.4		
Sylvia nisoria	3.0		
Sylvia curruca	2.1		

SUMMARY.

1. The graphing of the ecological relation between forest and shrubbery types of the area on the basis of qualitative distribution (See Table I., Figure 2.).

2. Calculation of the sociological values from repeated spring surveys of 19 stations (altogether 76 ha) as follows: density of the total population per km^2, abundance of the species, individual dominance of the species, dominance-groups. (Table III.).

Population studies made in one season and over a limited area are open to much criticism. I present the foregoing, however, for different reasons — for 1946 and for the Tihany peninsula they are accurate, and they give a good basis for methodical reflections, as follows:

3. Palmgren's quadrate method was sufficient in the open wood-

land and the more open shrubberies. The density of the closed forest and the large quantity of birds therein, on the other hand, caused great difficulties. Here it was necessary to repeat the survey 3—4 times, and to form small stations not more than 2 ha in extent.

4. The aspect of propagation (considering only the first breeding) extends to different epochs in the hibernating residents among the early arriving (beginning of April) and late (at the turn of April-May) summer residents. Therefore we have no standard period in which all members of the population are bound to their territories and thus easily countable. In April some dominant species are still absent; again, by the middle of May the earliest breeders have got their young out of the nests, thereby finishing their localised season. All these relations are explained by the phaenological spectra (Figures 4, 5).

5. The nesting and feeding territory is not the same place for the greater part of the species of the shrubby pasture and forest. In the sociological characterising of these we must express, with a special relative figure, that the species nests or feed, or both, locally, in the community individual recorded, or generally, that is, in the whole community. Because of these species I could not employ the term character-species („Karakterart" of the botanists) nor the degrees of Fidelity (exclusiveness).

6. This method is not suitable for calculating degrees of constancy. The correct establishment of constancy and frequency must take place in connection with the time factor — and attempts are being made in this direction — because the role of the bird is periodical in the different biotic communities.

Grateful acknowledgment is made to my colleague Dr. L. Felföldy, who assisted me with the figures and made map No. 1, as well as the photographs; to Mrs. J Thompson Vass for the English translation; to Mr. F. Holly for the meteorological observations and to Miss M. Fenyvesi for the other technical help.

REFERENCES.

Bacsó, N. (1932). *Magy. Biol. Kut. Munk.* **5.** 236.
Bacsó, N. (1933). *Magy. Biol. Kut. Munk.* **6.** 325.
Bacsó, N. (1934). *Magy. Biol. Kut. Munk.* **7.** 334.
Bacsó, N. (1935). *Magy. Biol. Kut. Munk.* **8.** 420.
Bacsó, N. (1938). *Magy. Biol. Kut. Munk.* **10.** 456.
Bacsó, N. (1939). *Magy. Biol. Kut. Munk.* **11.** 1.
Bacsó, N. (1940). *Magy. Biol. Kut. Munk.* **12.** 398.
Bacsó, N. (1941). *Magy. Biol. Kut. Munk.* **13.** 25.

FELFÖLDY, L. (1943). *Tisia* **6.** pp. 18.
GLASEWALD, K. (1939). Die Vogelwelt eines Laubwirtschaftswaldes der Mark Brandenburg. Neudamm & Berlin, J. Neumann.
HARTERT, E. (1903—22). Die Vögel des paläarktischen Fauna. I—III. Berlin, V. Friedländer.
HOMONNAY, N. (1938). *Magy. Biol. Kut. Munk.* **10.** 52.
KALELA, O. (1938). *Ann. Zool. Soc. Zool.-Bot. Fenn. Vanamo.* **5.** No. 9. pp. 291.
KEVE, A. & PÁTKAI, I. & VERTSE, A. (1943). *Magy. Biol. Kut. Munk.* **15.** 153.
KEVE, A. & PÁTKAI, I. & UDVARDY, M. v. & VERTSE, A. (1946). *Arch. Biol. Hung.* 17. p. 51.
LACK, D. (1937). *Ibis* 14. **1.** 369.
LINDSALE, J. M. (1936). *Wilson Bull.* **48.** 158.
MAPS: 1:25.000. *Tihany.* No. 5260 I.-c. Áll. Térk. Int.
1:20.000 Tihanyi Félsziget. *Magyar Fürdőtérképek.* **6.** Áll. Térk. Int.
1: 5.000. in: Papp, F. (1931). *Magy. Biol. Kut. Munk.* **4.**
PALMGREN, P. (1930). *Acta Zool. Fennica.* **7.** pp. 219.
PALMGREN, P. (1931). *Ornis Fenn.* **8.** 73.
PALMGREN, P. (1933). *Ornis Fenn.* **10.** 61.
PALMGREN, P. (1936). *Acta Zool. Fennica.* **14.** pp. 59.
PALMGREN, P. & Ahlquist, H. & Luther, F. (1938). *Ornis Fenn.* **15.** 120.
PÁTKAI I. (1942). *Magy. Biol. Kut. Munk.* **14.** 231.
RÉTHLY, A. (1939). *Magy. Biol. Kut. Munk.* **12.** 393.
SALOMONSEN, F. (1946). *Dansk. Orn. For. Tidsskrift.* **40.** 13.
SCHIERMANN, G. (1930). *Jour. f. Ornith.* **78.** 137.
SCHIERMANN G. (1934). *Jour. f. Ornith.* **82.** 445.
SCHIERMANN, G. (1939). *Jour. f. Ornith.* **87.** 181.
SOÓ, R. v. BERE (1931). *Magy. Biol. Kut. Munk.* **4.** 293.
SOÓ, R. v. BERE (1932). *Magy. Biol. Kut. Munk.* **5.** 122.
SOVERI, J. (1940). *Acta Zool. Fenn.* **27.** pp. 176.
SRÁMEK HUSEK, R. (1944). *Rozpr. II. Tridy Ceské Akad.* III. **17.** pp. 39.
STEGMANN, B. K. (1938). Principes généraux des subdivisions ornithogéographiques de la région paléarctique. *Fauna URSS. Oiseaux.* I. pp. 157.
STEINBACHER, G. (1942). Jour. f. Ornith. **90.** 342.
UDVARDY, M. v. (1941). *Tisia.* **5.** 92.
VÄLIKANGAS, I. (1937). *Ann. Ac. Sci. Fenn.* Ser. A. 45. No. **5.** pp. 236.
WUST, W. (1931). *Verhandl. Ornith. Ges. Bayern.* **19.** 223.

EXPLANATION OF TABLE III.

The quantitative data for each station and the values reckoned therefrom. () In parenthesis those of accidental character. The 4 rows at top of Table: A = data for all pairs found. B = data for only the pairs which bred on the area. Indications given in last row of upper section: I—VII = survey number. Σ = No. seen on each of the repeated surveys. Ab. = abundance (No. of pairs/km^2). D = Individual dominance (%ual role of each species, reckoned in pairs, of the entire bird population of the area.

Number and character of station given in top row of upper section: O = open woodland. F = closed forest. P = scrubby pasture. S = shrubbery. T = grassy hilltops.

TABLE III. The quantitative data for each station and the values reckoned therefor.

No. and charakter of Station	1. O	2. O	3. F	4. F	5. F	6. S—P	7. P	8. P	9. P	10. P—T
Date of the survey	V. 10.	V. 20.	V. 9, V. 11, V. 23, VI. 12.	IV. 9, IV. 26, V. 8, V. 11, V. 18, V. 23, VI. 12	IV. 26, V. 8, V. 11, V. 18, V. 23, VI. 12.	VI. 1, VI. 6.	V. 10, V. 15.	V. 14, V. 28.	V. 17, VI. 5.	V. 12.
Size of the area (ha).	7'38	9.33	0'75	1'91	0'88	2'27	15'83	16'95	16'33	6'54
Bird density pro 100 ha	469 414	1031 965	6143	2948	3076	3300 1716	504 411	1034 448	546 342	40 34
	A B	A B				A B	A B	A B	A B	A B
Total number in pairs	34 30	95 85	46	56	27	75 39	68 56	174 75	88 60	19 17
Total number of species	17 14	32 26	22	24	16	25 13	21 12	21 13	30 13	9 7
	I. Ab D I. Ab D	I. Ab D I. Ab D	I. II. III. IV. Σ Ab D	I. II. III. IV. V. VI. VII. Σ Ab D	I. II. III. IV. V. VI. Σ Ab D	I. II. Σ Ab D I. II. Σ Ab D	I. II. Σ Ab D I. II. Σ Ab D	I. II. Σ Ab D I. II. Σ Ab D	I. II. Σ Ab D I. II. Σ Ab D	I. Ab D I. Ab D
Corvus cornix	2 28 8'8 2 28 6'7	1 11 1'0 1 11 1'1	1 1 1 134 2'2	2 1 1 1 1 2 105 3'6		1 1 44 1'3	2 2 15 2'9	3 2 3 18 1'7	2 2 13 2'3	
Coloeus monedula		1 11 1'0 1 11 1'1			1 1 53 1'8			80 80 472 46'0		
Pica pica					1 1 53 1'8				1 1 6 1'1	
Garrulus glandarius			1 1 1 134 2'2	1 1 53 1'8						
Sturnus vulgaris	1 14 2'9 1 14 3'3	10 108 10'6 10 108 11'2								
Oriolus oriolus	1 14 2'9 1 14 3'3	5 54 5'3 5 54 5'6	2 2 1 2 2 267 4'3	4 4 3 3 3 4 210 7'1	2 1 2 2 1 2 228 7'4	1 1 44 1'3		1 1 6 0'6	1 1 1 6 1'1	
Chloris chloris			1 1 134 2'2	1 1 1 1 53 1'8	1 2 1 2 228 7'4	1 1 44 1'3	1 1 8 1'5	2 2 12 1'2	2 2 13 2'3	
Carduelis carduelis			2 1 2 267 4'3	1 1 1 1 1 53 1'8	1 1 1 44 1'3	2 1 2 88 2'7	1 1 8 1'5			
Carduelis cannabina						2 1 2 88 2'7	6 6 6 44 8'8			
Fringilla coelebs	5 68 14'7 5 68 16'7	11 118 11'6 11 118 12'4	5 6 6 5 6 800 13'0	1 2 4 3 3 3 4 210 7'1	1 1 2 2 1 1 2 228 7'4	1 1 1 44 1'3	6 6 6 44 10'1	4 3 4 24 2'3	4 4 4 25 4'6	1 3 5'3
Passer domesticus				(1) (1) 53 1'8		1 1 1 44 1'3	1 1 6 0'6	1 1 6 0'6	4 4 4 25 6'7	1 3 5'9
„ montanus		3 33 3'2 3 33 3'2		(2) (2) 105 3'6					2 2 13 2'3	
Emberiza citrinella	4 55 11'8 4 55 13'3	8 86 8'3 8 86 9'0	2 2 3 3 3 400 6'5	4 5 4 3 3 2 2 5 263 8'9	1 1 1 1 3 3 341 11'1	10 8 10 440 13'0	10 8 10 440 26'0	4 2 2	2 5 5 31 5'7	
Alauda arvensis								1 1 1 8 1'5	9 14 14 86 15'9	4 7 21'0
Anthus campestris		2 22 2'1 2 22 2'3						12 19 19 113 10'1	12 19 19 113 25'3	6 10 31'6
„ trivialis		1 11 1'0 1 11 1'1								1 3 5'3
Sitta europaea										4 7 23'5
Parus maior	3 41 8'8 3 41 10'0	4 43 4'2 4 43 4'5	3 3 2 2 3 400 6'5	3 3 4 3 5 3 3 5 263 8'9	2 2 1 2 228 7'4	1 1 44 1'3				6 10 35'3
„ palustris			1 1 134 2'2	1 1 53 1'8						
Aegithalos caudatus			1 1 134 2'2	1 1 53 1'8						
Lanius minor	1 14 2'9 1 14 3'3	1 11 1'0 1 11 1'1								
„ collurio	3 41 8'8 2 28 6'7	3 33 3'2 3 33 3'4	1 1 1 134 2'2	1 3 2 3 158 5'4	2 1 1 2 228 7'4	1 1 1 44 1'3	1 1 1 44 2'6			
Muscicapa striata	1 14 2'9 1 14 3'3	5 54 5'3 5 54 5'6	1 1 1 134 2'2							
„ hypoleuca	1 14 2'9	1 11 1'0								
Phylloscopus trochilus			(2) (2) 267 4'3		(1) (1) 114 3'7					
„ sibilatrix	(1) 14 2'9	(1) 11 1'0		(2) (1) (2) 105 3'6	(2) (2) 228 7'4					
Locustella naevia		(1) 11 1'0								
Acrocephalus palustris									1 1 6 1'1	
Hippolais icterina			1 1 134 2'2						1 1 6 1'1	
Sylvia nisoria	2 28 5'8 2 28 6'7							2 3 3 18 1'7	2 3 3 19 3'4	3 5 15'8
„ borin						3 3 132 4'0	3 3 132 7'7			
„ atricapilla		4 43 4'2 4 43 4'5	3 3 1 2 3 400 6'5	2 4 3 4 1 4 4 210 7'1	1 1 1 114 3'7					
„ communis			1 1 1 134 2'2			6 3 6 264 8'0	6 3 6 264 15'4	17 17 17 101 9'8	17 17 17 101 22'7	3 5 17'6
„ curruca				1 1 53 1'8	1 1 1 114 3'7			2 1 2 12 1'2	2 1 2 12 2'7	
Turdus merula	1 14 2'9	5 54 5'3 5 54 5'6	2 3 2 3 3 400 6'5	4 1 1 3 4 210 7'1	1 1 1 1 1 114 3'7			1 1 6 0'6		
Oenanthe oenanthe						2 2 88 2'7	2 2 88 5'1	2 2 12 1'2	2 1 2 13 2'3	1 3 5'3
Saxicola rubetra								4 3 4 29 5'9	1 1 1 6 1'1	1 3 5'9
„ torquata						1 1 1 44 1'3	1 1 1 44 2'6	7 6 7 51 10'0	5 4 5 31 5'7	
Luscinia megarhynchos	6 65 6'4 6 65 6'7	4 2 1 1 4 534 8'7	2 5 5 5 4 1 5 263 8'9		2 2 228 7'4	2 3 3 132 4'0	2 3 3 132 7'7	4 3 4 29 5'9	5 3 6 37 6'8	1 3 5'3
Erithacus rubecula	1 11 1'0 1 11 1'1			1 1 53 1'8				7 6 7 51 12'3	6 3 6 37 10'0	
Troglodytes troglodytes				1 1 1 53 1'8				9 3 9 54 5'2		
Hirundo rustica								9 3 9 54 12'0		
Delichon urbica						3 8 8 352 10'7		8 9 9 54 5'2		
Apus apus						15 10 15 660 20'0		8 9 9 54 12'0		
Upupa epops	1 14 2'9 1 14 3'3	2 22 2'1 2 22 2'3	1 1 1 1 134 2'2			4 4 176 5'3				
Coracias garrulus		1 11 1'0 1 11 1'1				1 1 44 1'3	1 1 1 8 1'5	1 1 6 0'6	2 2 13 2'3	
Picus viridis		1 11 1'0 1 11 1'1								
Dryobates maior		1 11 1'0 1 11 1'1							1 1 6 1'1	
Jynx torquilla	1 14 2'9 1 14 3'3	1 11 1'0 1 11 1'1	1 1 134 2'2	1 1 53 1'8	1 1 114 3'7					
Cuculus canorus		2 22 2'1 2 22 2'3								
Falco cherrug									1 1 1 6 1'1	1 3 5'3
„ subbuteo	1 14 2'9 1 14 3'3					1 1 44 1'3				
„ tinnunculus						1 1 44 1'3	1 1 8 1'5		1 1 6 1'1	
Buteo buteo		1 11 1'0					1 1 8 1'5			
Accipiter gentilis		1 11 1'0							1 1 6 1'1	
Columba oenas		1 11 1'0 1 11 1'1				1 1 44 1'3				
„ palumbus						1 1 44 1'3				
Streptopelia turtur	5 68 14'7 5 68 16'7	7 76 7'4 7 76 7'9	2 2 1 2 267 4'3	1 1 1 1 53 1'8						
Perdix perdix			4 4 5 5 5 667 10'9	4 2 3 4 4 4 210 7'9	1 3 2 3 2 2 3 341 11'1	5 3 5 220 6'7	5 3 5 220 12'8	3 3 18 1'7	1 2 2 13 2'3	
Sylvia sp.		1 11 1'0			1 1 114 3'7				1 1 6 1'1	
Sp.		2 22 2'4 2 22 2'3				3 3 132 4'0	3 3 132 7'7	1 1 8 1'5	1 1 8 1'8	1 1 6 1'7
Number in pairs MM	34 469 30 414	95 1031 85 965	37 30 29 26 46 6143	16 23 31 26 36 24 27 56 2948	15 15 9 10 9 8 27 3076	64 44 73 3300 38 21 39 1716	57 60 68 504 51 53 56 411	155 76 174 1034 62 65 75 448	67 73 88 546 55 50 60 342	19 40 17 34
Number of species	17 14	32 26	17 12 16 11 22	9 10 9 10 15 12 12 24	12 10 7 5 7 5 16	23 12 25 13 8 13	14 19 21 11 12 12	15 18 21 11 12 13	23 24 30 13 11 13	9 7



Figure 6.



Figure 7.



Figure 8.



Figure 9.

EXPLANATIONS OF FIGURES 6.—9.

Figure 6. — One type of open woodland from station 2. In the hollow of the *Acer campestre* in the foreground the *stock-dove* nested. Otherwise the part of the area to be seen here is the nesting territory of the *chaffinch, starling* and the *great titmouse,* and the clearing in the background of the *tree pipit.* (Photo: FELFÖLDY.)

Figure 7. Scrubby pasture from station 2. In the *rose* and *hawthorn* thickets in the foreground *nightingales, whitethroats, yellow buntings* nested. The background of rocky-grassy hilltops is the nesting territory of the *wheatear* and the *sandpipit.* (Photo: L. FELFÖLDY.)

Figure 8. The more open type of scrubby pasture. In the middle distance badly gnawed and trampled *wild rose* and *hawthorn* bushes; in the foreground *Festucetum pseudovinae* clearing with *Ononis spinosa.* Nesting place of the *Finnnet* and the *stonechat.* (Photo: L. FELFÖLDY.)

Figure 9. A more enclosed part of scrubby pasture, from the standpoint of bird ecology a transition to the shrubbery *Querceto-cotinetum.* In the background *oaks,* in the foreground thickets of *wild rose* and *hawthorn.* Breeding territory of *chaffinch, yellow bunting, white-throat, common warbler* and *red-backed shrike.* (Photo: L. FELFÖLDY.) All potographs made November 13th, 1946.

ZUR SYSTEMATIK UND SOZIOLOGIE DER PHANEROGAMEN VEGETATION DER UNGARISCHEN BINNENGEWÄSSER. NACHTRÄGE.*

Von: Prof. Dr. R. v. Soó (Debrecen)

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Im I. Teile meiner Untersuchungen habe ich die Literaturangaben der darin bearbeiteten Gattungen *(Callitriche, Ceratophyllum, Myriophyllum, Hippuris* und *Utricularia)* über ihre Vorkommen in (historischem) Ungarn nicht mitgeteilt, wie vom II. Teile *(Potamogeton)* ab. Zur Ergänzung dieses Mangels, gebe ich hier in gleicher Form, die Angaben über die nicht erwähnten Standorte sowie das ganze bezügliche Schrifttum. Natürlich ist auch in den späteren Fortsetzungen die neuere Literatur (seit 1934—6 bzw. 1938) nachzuholen, besonders die der neueren ungarischen Florenwerke, vor allem von Balázs (Meszesgebirge AGH. IV. 1942 und Nagykároly Scripta Bot. Mus. Transs. II. 1943), Boros (Donau-Theiss Zwischenstromland BK. XXXIII., Thermalgewässer BK. XXXIV. usw.), Horvát (Mecsek- Külsősomogy Borbásia IV.), Hargitai (Nagykőrös, Diss.), Kiss (Hegyalja BK. XXXVI.), Nyárády-Soó (Kolozsvár), Polgár (Győrmegye BK. XXXVIII), Rédl (Bakony), Soó-Máthé (Tiszántúl), Soó-Hargitai (Sátorhegység BK. XXXVII.), Soó (Mátra, Székelyföld I—II, Nyírség BK. XXXI—XXXIX., Bükk BK. XL.), Zsák (BK. XXXVIII), ferner Angaben der neueren rumänischen und tschechoslowakischen Literatur.

Ebenso gebe ich zum Schluss eine Aufzählung der Herbarexemplare unserer neueren Sammlungen, auf Grund der Herbarien der Univ. Kolozsvár und Debrecen, des Museumsherbars zu Kolozsvár und des Herbars von Dir. Dr. Á. Boros, dem ich — für Überlassung seiner neuen Forschungsergebnisse — hier meinen besten Dank ausspreche.

* Zu den Teilen I—IV. I. Magy. Biol. Kut. Munkái II (1928) 45—79. —II. in VII. (1934) 135—153. —III. (1936). in VIII. 223—240. —IV. in X. (1938). 174—194. Abgeschlossen Mitte 1943.

I. CALLITRICHE.

Da die Bestimmungen in der früheren Literatur entweder unzuverlässig *(C. verna)* oder gar falsch *(C. stagnalis, platycarpa, hamulata autumnalis)* sind, hat eine Zusammenstellung der Angaben wenig Sinn. Doch habe ich es versucht und ersieht man daraus, dass die *Callitrichen* in den ganzen Karpathen, im Mittelgebirge und in Transdanubien häufig, im Tieflande spärlich zu nennen sind. Die Mehrzahl der Daten bezieht sich gewiss auf *C. polymorpha*, bes. die Mitteilungen der für *„stagnalis"* oder *„hamulata"* bestimmten Exemplare.

So werden diese angegeben aus den Nordkarpathen (z. B. Hazslinszky Észak-Magyarhon. 278, Holuby ÖBZ. XIX. 378, Márkus ÖBZ. XV. 306, Sag.-Schneid. Centralkarp. 468, Filarszky Pieninen 51, Mauritz Göllnicv. 25, Zapalowicz Babiagora 91 usw.)
von Nordosten (Vagner Mármaros 161, Borb. MOTV. XIX. 145, Zapal. Rosl. Pok. Marm. 165, Margittai MBL. XV. 82, Hruby Bot. Arch. 1925. 244, 259 usw.)
aus Siebenbürgen meist als C. **transsilvanica** (vgl. Simk. Erdély 231—32 „sehr gemein", Simk. MTK. XVI. 107, Moesz Brassó főreál. ért. 1902 usw.)
aus dem Kom. Bihar (Steffek ÖBZ. XIV. 174, Simk. Nagyvárad 77, MTK. XVI. 107), Arad (Simk. Aradm. 125, Pauca Moma-Codru 1935:34), aus dem Banate (Heuffel ZBG. VIII. 106, Simk. MTK. XV. 540, MNL. VI. 20, Borbás Temesm. 29 usw.), aus dem Borsoder Bükkgebirge (Reuss Kvetna Slov. 154, Budai MBL. XII. 322), der Umgebung von Pozsony (Lumnitzer Fl. Poson. 2, Kornhuber I. 73), dem Kom. Baranya (Simk. MTK. XI. 200), Sopron und Vas (Gombocz Sopron 108, Borb. Vasm. 175, Waisb. Kőszeg 2, Gáyer Vasm. Muz. Évk. II. 254) Die **C. autumnalis** — Angaben beziehen sich wohl auf sterile Formen der **C. verna** oder **polymorpha**, mit schmalen untergetauchten Wasserblättern.
Sichere **C. polymorpha** Angaben sind diejenigen nach dem Erscheinen meiner Bearbeitung, so aus dem Kom. Győr (Polgár BK. XXXVIII. 289: Győr, Győrsziget, Mórichida-Rábaszentmiklós), dem Mátragebirge (Soó Mátra 48), dem Bükkgebirge (Soó BK. XL. 199), dem Sátorgebirge (Kiss BK. XXXVI. 234, Soó BK. XXXVII. 182: Pálháza, Sárospatak, Pusztafalu), dem Meszes (Balázs AGH. IV. 172, vgl. die Aufzählung neuer Standorte), von Kolozsvár (Nyárády-Soó Kolozsvár 353), dem Seklerlande (Soó Székelyföld 79), aber auch vom Tieflande (Kom. Arad und Bihar, auf Grund älterer Mitteilungen: Soó Tiszántúl 122), aus der Nyírség (Boros Nyírség 93, Soó BK. XXXI. 238: Vállaj, Nyírpilis, Hajdusámson) In der Flora des Bakonygebirges von Rédl fehlt sie, obwohl von mir schon früher (MBIM. II. 63) vom Kabhegy angegeben wurde. Dieselben Werke zählen auch immer sichere **C. verna** auf, ebenso noch Horvát aus dem Kom. Baranya (Mecsek fl. 104)

Da **Callitrichen** im Alföld selten sind, seien noch hier weitere **C. verna** Angaben aufgezählt. **Titelicum:** Baranya, Albertfalu (Horvát BK. L. 107), Ujvidék, Kabol, Káty (Zorkóczy Ujvidék 95), Óbecse (Kovács Óbecse 120), Pancsova (Simk. MNL. VI. 20. **„hamulata"), Samicum:** ausserhalb der Nyírség noch Kál-

mánd, Gilvács (Balázs Scr. Bot. Mus. Transs. II. 19), **Crisicum:** vgl. Soó-Máthé Tiszántúl 122. **Arrabonicum:** ausser Pozsony (s. oben) noch P. szentgyörgy (Kornhuber PV. 1858. 33), bestimmt auch anderswo.

Der gültige Name von *C. verna* L. (1755) ist — nach den neuen Nomenklaturregeln — *C. palustris* L. (1753. p. maj. p.).

II. CERATOPHYLLUM L.

1. C. submersum L.

Budapestini: Veresegyháza (Boros BK. XIX. 42).

Praemetricum: Tápióvölgy: T. szele, T. bicske, Nagykáta (Kern. Vegetverh. 167), Akasztó (Menyhárt Kalocsa 8), cf. Lányi MBL. XIII. 254.

Titelicum: Ferenc- és Ferenc József-csatorna (Prodan MBL. XV. 219) Ujvidék, Kabol, Káty (Zorkóczy 93), Óbecse (Kovács 79), cf. Borb. Temesm 29, Simk. MTK. XI. 200.

Samicum: Ujdávidháza, Várpalánka, Gát (Margittai MBL. X. 396,) Debrecen, Ujléta, Kállósemlyén (Soó BK. XXXI. 232, XXXVI. 309), Igrice, Nyírbéltek, Pátroha (Boros Nyírség 74) Ujfehértó, Nagyhalász (Zsák BK. XXXVIII. 20), cf. Borb, Szabolcs 257, Boros 1. c.; Csanálos, Mezőfény (Balázs Scr. Bot. II. 14.)

Crisicum: Hajdunánás (Ujvárosi Debr. Sz. X. 60). Szentanna (Simk. Aradm. 125), Rakamaz, Konyár (Boros ap. Soó Tiszántúl 81), Püspökladány (Zsák 1. c.) Hajduszoboszló (Soó Debreceni Szemle 1940, 162), Torontál m. Makó „Ladányi ér" (Győrffy MBL. IV. 283) cf. Budai MBL. XV. 261.

Matricum: Esztergom, Tata (Feichtinger Esztergom. 243), Sárisáp (Feicht. MOTV. 1864. 284), cf. Soó Mátra fl. 25,

Arrabonicum: Sopron „Tómalom", a Fertőnél (Szontágh ZBG. XIV. 278, Reich. ZBG. XI. 339, Gombocz Sopron 98), Hanság (Wierzb. ap. Neilr. 75), Győr, Győrsztiván, Gy.-sövényháza, Lébény, Kismegyer (Polgár BK. XXXVIII. 264).

Transdanubicum: Kisbalaton, Zalafolyó, B. boglár (Borbás Balaton 330 cf Soó MBIM. X. 199).

Transsilvanicum: Váradszőllős—V. sztmárton (Simk. MTK. XVI. 107), Torda, Kőhalom (Baumgt. En. II. 90), Tövis, Vízakna, Szterzsébet (Fuss Transs. 220), Nagyszeben (Schur En. 217), Segesvár (Fronius Progr. Gymn. Schässb. 1858. 32), Rétyi Nyír (Moesz MBL. IX. 344), Kolozsvár, Apahida (Nyár. BK. XXXVIII. 136, Nyár.-Soó Kolozsvár 208). Nach Prodan in allen Seen der Mezőség MBL. V. 32), selbst sah ich in Hodostó (Cege) nur **C. demersum.**

Var. Haynaldianum wird von Borbás mit dem Typus zusammen von Temesvár und Pozsony angegeben (MNL. VIII. 20, IX. 38, ÖBZ. XXXIV. 182)

2. C. demersum L.

Im Tieflande und in Transdanubien häufig, deshalb werden die Standorte nicht einzeln aufgezählt. **Budapestini** cf. Borbás Bpest 182, Tőkés Vác 38. — **Praematricum** cf. Kerner 167, Menyhárt Kalocsa 80, Boros BK. XXXIII. 89. — **Titelicum** cf. Feicht MTK. VIII. 19, Prodan MBL. XIV. 219, Zorkóczy

Ujvidék 95, Kovács Óbecse 79, Simk. MTK. XI. 200, Boros MBL. XXI. 71, XXIII. 35, Horvát Baranya 72, Simk MNL. VI. 19 (Pancsova), Borb. Temesm. 29 (Ujarad), Tőkés Temesvár 21, Tuzson Alföld 146 (Kevevára). **Crisicum:** cf. Soó-Máthé Tiszántúl 81. — **Samicum:** nordwärts bis zu den Kom. Zemplén—Bereg cf. Kiss BK. XXXVI. 212, Chyzer MBL. IV. 317, Hargitai AGH. II. 148, Margittai Kvartalnik 57, in der Nyírség cf. Boros Nyírség 74, Soó BK. XXXIV. 38, XXXIX. 309, Balázs Scr. Bot. II. 14.

Arrabonicum: cf. Lumnitzer 432, Kornhuber I. 73 ZBG. XXXV., Wiesbaur PV. 1871. 16, Polgár BK. XXXVIII. 264, bis zum Marchfeld (Degen-Gáyer MBL. XXII. 82)

Transdanubicum: bes. in Kom. Vas (Borb. Vasm. 175, Waisbecker Kőszeg 17), Sopron „Tómalom" (Gombocz 97), Balatongebiet (Borb. Balaton 330, Soó MBIM. X. 200), bis zu Simontornya (Boros MBL. XXIII. 17), Szekszárd Hollós BK. X. 101).

Seltener im Mittelgebirge. **Matricum:** Bakony Sárosfő (Rédl Veszprémi gimn. Ért. 4) Papkeszi (Bartha ap. Rédl Bakony 72), Kom. Esztergom öfters Kerner 167, Feichtinger MOTV. 1. c, Esztergomm. 243), Tata (Boros BK. XXXIII. 87), cf. Borb. MNL. IX. 39.

Transsilvanicum: nordwärts bis Huszt (Vagner Mármaros 165), in Siebenbürgen cf. Simk. Erdély 233, ferner Váradsztandrás (Simk. MTK. XV. 107), Apahida (Ujvárosi in Kolozsvár 654), Alsószovát (Borb. TTK. 1888. 38), Brassó (Moesz MBL. IX. 347), Vízakna (Csató Alsó-Fehér 25), nach Prodan (MBL. 1. c.) in allen Seen der Mezőség. Die von Borb. (MNL. IX. 41) von Fenyőfalva mitgeteilte var. **carinatum** ist mit der **var. polycanthum** von Schur identisch, vom letzteren (Enum. 218) publiziert: Vízakna, Szakadát.

Angeblich auch in den Nordkarpathen, Kom. Túróc (Pterikovic Sborn. Slav. Mus. XVII. 1912. 136)

III. MYRIOPHYLLUM.

1. M. verticillatum L.

Budapestini cf. Borbás Bpest 157, Kerner 166, Boros BK. XVI. 117. XXIV. 74. — Vác (Kernerl. c., Tőkés Vác 59) — **f. pectinatum:** Csepelsziget Borb. TTK. 1879. 29, Értek. termtud. IX: no. 16. 29).

Praematricum: Kalocsa „Vajas" (Menyhárt 79), Ócsa (Boros XXXIII. 90 et **f. pectinatum**), Tápiószele (Borb. ÖBZ. 1892. 288)

Titelicum: Zombor, Bezdán, Sajkáslak (Prod. MBL. XIV. 243), Kiszács (Kupcsok MBL. XIII. 90), Óbecse (Prod. 1. c., Kovács 130), Ujvidék, Káty (Feicht. MTK. VIII. 19, Zorkóczy 27) Mohácsi sziget „Riható" (Horvát Mecsek 112), Albertfalu (Horvát BK. XL. 108), Dárda (Knapp ap. Horvát 1. c.) cf. Simk. MTK. XI. 169, Boros MBL. XXIII. 42.

Samicum: Sárospatak Longierdő (Kiss BK. XXXVI. 241), Szamosszeg (Felföldy ap. Soó BK. XXXIX. 53), Várpalánka (Marg. MBL. X. 403), cf. Borb. Szabolcs 258, Boros Nyírség 97.

Crisicum: cf. Soó-Máthé Tiszántúl 131 viele Standorte, von Arad schon von Hazsl. MTK. X. 14 angegeben.

Arrabonicum: Pozsonyszentgyörgy Sur (Kornhuber P. V. 1858. 34), Kom.

Nyitra häufig (Knapp ZBG XV., 167, et **f. pectinatum:** Emőke, et **f. limosum:** Céteny, l. c. 168), Kom. Győr häufig (Polgár BK. XXXVIII. 297 et **f. pectinatum:** Győrsziget, Kulcsod), Guta, Dolos Derka (Krist Sborn. Brno XXII. 5) Illmic (Neumayer ZBG. 1929. 373) cf Lumnitzer 433, Ebenhöch 102 etc.

Vindobonicum: Morvamező: Szakolca (Holuby P. V. II. 83), Detrekőcsütörtök-Malacka (Degen-Gáyer MBL. XXII. 96).

Matricum: Sárosfő (Rédl Bakony 103), Párkány, Nána, Tokod, Kural, Tata (Kerner 166, Feicht. Esztergom 243), Sajóarnót (Budai ap. Soó BK. XL. 201).

Transdanubicum: Sopron "Tómalom" (Szontagh ZBG. XIV. Gombocz Sopron 121) Kom. Vas häufig (Borbás Vasm. 276), Celldömölk, Izsákfa (Gáyer in notis), Keszthely (Szenczy ap. Borb. Balaton 409), Szólád (Mágocsy BK. XIII. 127) Badacsony-Ábrahámhegy (Borb. l. c. et **f. pinnatifidum),** cf. Soó MBIM. X. 201 Gárdonypuszta (Horváth Borbásia IV. 47), Balatonhidvég (Boros MBL. XXIII. 42. cf. Boros MBL. XXIII. 17.

Carpaticum: Bohuslavice, Rovne (Holuby Trencsén 193), Arvanagyfalu, Oravka (Szontágh ZGB. XIII. 1093), Poprád (Scherfel ex Sag.-Schneider 182).

Transsilvanicum: cf. Simk. Erdély 132, Soó Székelyföld 87, Suppl. 37 (Nyárádtő, Marosvásárhely, Alcsiki medence) Nyárády-Soó Kolozsvár 385, Prodan MBL. V. 32—?) **f. limosum:** Apahida-Szamosszentmiklós (Soó Kolozsvár 663), **f. pectinatum:** Nagyszeben (Schur Enum. 215) Nagyvárad-, Fugyivásárhely (Simk. MTK. XVI. 107. Nagyvárad 160), Belényes-Petrani (Kerner 166), Brassó-Kereszténytalva (Borb. Értek. termtud. IX. no. 16. 29), Mármarossziget (Borb. MOTV. XIX. 155).

2. **M. spicatum** L.

Budapestini cf. Borbás Bpest 157, Kerner 166.

Praematricum: Nagykőrös (Hargitai 40), Hajós, Dusnok, Kecel, Dunapataj, D.-földvár, Kömlőd (Menyhárt 79), Dabas (Tuzson Alföld 165), Kiskundorozsma, Szeged (Lányi MBL. XIII. 263. cf. Tuzson l. c. 165) cf. Menyhárt l. c., Boros BK. XXXIII. 90.

Titelicum: Bácska, wie das M. verticillatum (Feichtinger, Prodan, Kupcsok, Zorkóczy, Kovács l. c.) Temesvár: Giroda, Mehala (Borb. Temes 74, Tökés Temesvár 34) Kevevára (Tuzson Alföld 146) — cf. Simk. MTK. XI. 169, Boros MBL. XXI. 71.

Samicum: Sárospatak (Kiss BK. XXXVI. 241), Longerdő (Kiss l. c., Hargitai AGH. II. 148), Ágcsernyő (Chyzer MBL. IV. 323), Alsódomonya (Bucek Sborn. Brno 1931. 94), Nyirmada, Kántorjánosi (Boros Nyirség 97), Ujfehértó-Érpatak, Hajdubagos (Soó BK. XXXIV. 40).

Crisicum: zahlreiche Standorte s. Soó-Máthé Tiszántúl 131.

Arrabonicum: Pozsonysztgyörgy Sur (Kornhuber P. V. 1858. 34), Kom. Győr häufig (Polgár BK. XXXVIII. 297), Hanság (Kornh. cf. Gombocz Sopron 121) cf. Lumnitzer 433.

Vindobonicum: Zohor, Drösing, Nagylévárd (Degen-Gáyer MBL. XXII. 96).

Matricum: Tokod, Kural (Feicht. Esztergom 243), Sajókirályi (Boros BK. XXXIV. 102), Görömbölytapolca (Soó BK. XI. 201) cf. Kerner 166, Feicht l. c., Boros l. c. 87.

Transdanubicum: Sopron, Ruszt, Kismarton (Stur, Uhl ex Gombocz Sopron 121), Kethely (Waisb. Kőszeg 58), Celldömölk, Vasvár (Borb. Vasm. 276), Szekszárd (Hollós BK. X. 106), Simontornya (Pill. ap. Horvát Borbásia IV. 47), Somogycsicsó (Boros MBL. XXIII. 42), in Balatonsee u. d. Umgebung, (cf. Borb. Földr. Közl. 1891. 470, Balaton 73—75, 409, Soó MBIM. X. 201).

Carpaticum: Pöstyén (Domin Piestany 171), Árva m. Bobró (Vitkay ap. Szontagh ZGB. XV. 1093), Kom. Szepes (Mauksch ap. Wahlbg. 308 ex. Sag.-Schneid. 182), Pieninen: Dunajec (Filarszky 32) cf) Holuby Trencsén 193, Moesz BK. X. 174.

Transsilvanicum: Czornahora (Hruby Bot. Arch. 1925. 259)? — Tövis (Csató Alsó-Fehér 25), Cege (Györffy MBL. 164), alle Seen der Mezőség (Prodan MBL. V. 32)? Rétyi-Nyir (Moesz MBL. IX. 345), Szászfenes, Szamosfalva, Dezmér (Nyárády-Soó Kolozsvár 385, 664), Rév, Barátka, Várson-kolyos (Freyn MTK. XIII. 124), Belényes-Petrani (Kerner l. c.) weitere Standorte cf. Simk. Erdély 232.

IV. HIPPURIS VULGARIS.

Budapestini: cf. Sadler Fl. com. Pest 7, Kit. Ann. Mus. Nat. XXVI. 186. Kerner 166, Filarszky TTK. 1894. 117, Degen MBL. IV. 22 (Soroksár), Pénzes MBL. XXXII. 88 (Lágymányos); Vác (Tőkés 59).

Praematricum: Dunaföldvár, Dusnok-Hajós (Menyhárt 79).

Samicum: cf. Boros Nyirség 97 — **Crisicum:** Szolnok (Kerner 166), Szikra (Hollós Kecskemét 103) — **Titelicum:** Bezdán, Dunaszigetek (Prodan MBL XIV. 243, Tuzson l.c.), Káty (Zorkóczy 27), Óbecse (Kovács 139), Temesvár (Tőkés 34), Baranya m. Laskó (Simk. MTK. XI. 169) cf. Simk. l. c., Boros MBL. XXI. 71.

Arrabonicum: cf. Lumnitzer l. c. — Hanság (Kornhuber ZBG. XIV.), Pozsonyszentgyörgy "Sur" (Kornhuber PV. 1858. 34) Fertő (Wallner Soproni ért. 1903. 19), Kismarton (Uhl ap. Gombocz Sopron 121), Győrszentmárton, Koroncó (Ebenhöch P. V. 1860. 102), um Győr, Győrrévfalu häufig (Polgár Ért. 1903. 23, BK. XXXVIII. 297), Komárom m. Gyulamajor, Nagymegyer, Bős, Dolos Dorka (Krist Sbornik Brno XX. 5, XXI. 6, XXII. 4, 5), Nána, Párkány (Feichtinger MOTV. 1864, Esztergom. 242), Herkály, Dunaalmás, Szőny, Neszmély (Hillebrand ÖBZ. 1858. 299).

Vindobonicum: Marchfeld Abrod, Zohor (Degen-Gáyer MBL. XXII. 96), Szakolca (Holuby ap. Knapp ZBG. XV. 167).

Matricum: Tata (Feichtinger l. c.)

Transdanubicum: Keszthely (Szenczy ap. Borbás Balaton 409), Kéthely, Fenékpuszta (Borb. l. c., Mágocsy BK. XIII. 120), Kisbalaton (Soó MBIM. II. 136) Siófolyó, Mezőkomárom (Kit. Addit. 276, Ann. Mus. XXVI. 186) — Celldömölk, Izsákfa (Gáyer in notis), Kemeneskápolna (Jeanplong Borbásia II. 59) — cf. Borbás l. c. TTK. 1894. 496, Vasm. 276, Soó MBIM. X. 201.

Carpaticum: Rozsnyó (Fábry Gömör vir. 8), Bohuslavice (Holuby Trencsén 193), Rovnye (Rochel ex Holuby l. c.), Laziszkó Liptó m. (Mihalik MKE. XIII. 24), Szepesbaldóc (Répászky ad Jáv.)

Transsilvanicum: Huszt (Vagner Mármaros 179), Nagyszeben (Schur En.

216), Szentágota, Kőhalom (Baumgt. Enum. 166) cf. Janka MTK. XII. 186. Györffy MBL. III. 165. (Moesz MBL. IX. 347).

V. UTRICULARIA.

1. U. vulgaris L.

Im ganzen Gebiete der pannonischen Flroa, wenn auch zerstreut, verbreitet, deshalb habe ich auf die Aufzählung der Standorte auch bei den Herbarexemplaren verzichtet, und nur die Komitate erwähnt, woher ich gesehen habe. Seltener ist sie in den Gebirgen.

Matricum. Sárosfő (Rédl Veszprémi gimn. ért. 1930. 2) Veszprém. (Kit. ex Jáv. Ann. Muss. Nat. 1936. 93), Tata (Boros BK. XXXIV. 87) Esztergom, Nána, Kövesd (Feicht. 120) Szentendre (Kerner 119, Borb. Bpest 119), Vác (Tőkés 40), Gyöngyös (Soó Mátra 69), Egerbakta (Boros MBL. XXIII. 63), Kisgyór-Harsány (Budai MBL. XII. 325).

Carpaticum: Bohuslavice, Rovne (Holuby Trencsén 152), Lucski (Mihalik MKÉ. XII. 32), Árvanagyfalu (Vitkay ap. Szontagh ZGB. XV. 1077), Oszada (Szontagh l. c.) Pieninen: Alsólechnitz (Filarsky 46), Rimaszombat (Fábry Gömörm. v. 5). Am Marchfeld und in Kom. Neutra. schon Haufiger (Holuby P. V. II. 83, Krzisch 86, Degen-Gáyer BK. XXII. 108) in Nordsten schon selten, so Bátyu, Munkács (Marg. MBL. X. 408).

Transsilvanicum: viele Standorte cf. Simk. Erdély 456, Soó Székelyföld 114, Nyárády-Soó Kolozsvár 492, ferner Gyulafehérvár (Cserni Alsófehérm. évk. IX. 31), Tövis, Verespatak (Csató Alsó-Fehér 56), Cege (Prod. MBL. V. 32).

2. U. neglecta Lehm.

In der Literatur kaum erwähnt.

Crisicum: Tiszaroff (Perlaky ÖBZ. XL. 1890. 208).

Carpaticum: Szepes m. (Kalchbr. ap. Hazsl. Északi Magyarh. 169 sub **U. intermedia).** Fraglich: Kelemér Mohos (Boros MBL. XXIII. 64).

Arrabonicum: Győr m. Dunaszeg (Polgár BK. XXXVIII. 320).

3. U. minor L.

Arrabonicum: Magyaróvár (Vuezl ap. Neilr. Aufz. 195) Nezsider, Nyulas (Gombocz Sopron 155).

Transdanubicum: Kismarton (Uhl ap. Gombocz l. c.), Felsőőr-Alsólő (Pauer Vasm. term. eml. 60), Lesenceistvánd (Gáyer MBL. XXIII. 58: **Bremii).**

Matricum: Tata (Boros BK. XXXIV. 87).

Carpaticum: Alsótátrafüred (Czakó MKÉ. XV. 152), Borylápok (Nyár. BK. X. 7 sub **U Bremii).**

4. U. Bremii Heer.

Budapestini: Óbuda (Simk. MNL. 1877. 148, Borb. Bpest. 119).

Praematricum: Ócsa, Izsák (Boros BK. XXXIV. 90).

Crisicum: Aszód-Tiszaföldvár (Haberle ap. Kit. ZGB. XIII. 79. sub **"U. minor"?)**

Samicum: Szernye: Fornos (Boros Honism. Biz. Közl. No. 5. 5) Kállósemjén (Boros Nyírség 117)?, Nyírmada (Soó BK. XXXI. 246)?

Vindobonicum: Detrekőcsütörtök—Malacka „Umicenka" (Scheffer in Degen-Gáyer MBL. XXII. 108).

Transdanubicum: Villány Simk. MTK. XI. 183 sub **"U. minor").**

Transsilvanicum: Kolozsvár (Landoz Erd. Muz. II. 35): Szelicse (Soó Geob. Mon. Kolozsv. 81), Apahida (Soó BK. XXII. 76), Diósi tó, Kajántó (Nyárády Kolozsvár 492 Erd. Muz. 1941. 184) von „Városlója" ist zu streichen, da Knapps Pflanze: **U. vulgaris!** Felsőárpás (Schur Enum. 548), Büdös: Kukojszás (Schur VSV. X. 198), Rétyi Nyír (Moesz BK. X. 133, aber in MBL. IX. 346 als fehlendes erwähnt, Boros ap. Soó Székelyf. suppl. 47), Szászhermány (Römer VSV. LXI. 43).

5. **U. biflora** Lam.

Keszthely: Hévíz (cf. Boros BK. XXXIV. 96, Soó MBIM. X. 202).

ERGANZUNGEN ZU DEN HERBAREXEMPLAR-STANDORTSANGABEN DER TEILE I—IV.

I. CALLITRICHE. **1. C. palustris** L. (Syn. C. verna L.)

Debrecen (Papp)?, Lillafüred, Tapolcafürdő (Soó), Felsőtárkány (Boros, Soó), Ómassa „Disznóspatak", Kékes „Pisztrángostó" (Boros) Helpa Gömör m. (Kalchbrenner), Glaser-Hai Turóc m. (Wagner) Perecseny, Nevicke Ung m. ((Ubrizsy), Pósaháza, Ignéc (Ujvárosi et Udvardy) Kőszeg (Soó), Csákvár „Gémhegy" (Boros) Kőrösladány-Gyoma (Buchinger) Biharfüred (V. Borza), Bogdánháza (Soó et Balázs), Kolozsvár (Wolff, „Elővölgy" (Nyárády, Soó), Hosszuaszó (Barth), Magyarbagó (Pávai), Ünökő tavai (Czetz), Cisia (Porcius), Gyergyócsomafalva (Boros), Brassó (Moesz), Hohenstein (Römer), Oláhfalu (Soó), Petrozsény (Knapp).

2. C. polymorpha Lönnr.

Hajdusámson (Soó, Máthé), Nyírpilis „Uj tanya" (Soó), Pálháza „Kemencepatak" (Soó), Putnok „Szörnyi-v.", Parád „Feketető, Kőrösmocsár" (Boros), Lesenceistvánd, Győrsziget, Mórichida—Rábaszentmiklós, Hárságypuszta (Polgár) Mármaros Czarnahora „Breskul" (Boros), „Hermanieska, Hoverla" (Soó), Kolozsvár (Landoz sub **C. verna**, Béltéki, Soó), Felek (Soó), Szelicse (Nyárády) Topánfalva „Szohodol" (A. Richter), Ördögkut, Csucsa-Kissebes, Bádok (Soó) Borsa (Coman), Radna (Porcius sub **C. aestivali**), Radnaborberek (Rigler), Borszék (Soó), Hargitafürdő (Boros), Lucsmelléke (Nyár. FRE. 1286 sub **C. hamulata!**), Kimpulujnyág (Nyár. FRE. 1287 sub **C. verna!**)

Sterile Exemplare (?): Bélaer Tátra: Hátsó Rézaknák, Aggtelek, Sajtókirályi Salgótarján (Boros), Parád (Igmándy), Diósgyőr (Máthé), Szenic (Branik), Csütörtök, Bohuslavice (Holuby), Remete, Gyergyóalfalu (Soó), Korond (Nyár.), Marossólymos (Somogyi).

II. CERATOPHYLLUM. **1. C. submersum** L. Debrecen, „Haláp", Ujléta, Kállósemjén, (Soó), Hajdunánás (Ujvárosi), H. szoboszló (Fäller), Nyírmada (Boros), — Lepsény (Soó), Győr **(f. granulosum)**, Kismegyer (Polgár), Velencei tó. Dinnyés, Pákozd, Nagysziget (Boros) — Kolozsvár „Elővölgy" „Városlója" Apahida, Szind (Soó, Nyárády), Nagyenyed (Pávai).

**

2. **C. demersum** L. Ohat „Herep" (Soó, Máthé), Nyirmada, Érpatak, Sárospatak (Soó), Kék (Tatár), Tiszadob, T. dada, Hajdunánás (Ujvárosi). Pocsaj, Váncsod, Érkeserü, Doboz, Szikra, „Tős" (Máthé), Dévaványa, Kisujszállás (leg.?), Tarnaörs (Tauscher), — Neszmény, Pákozd, Várpalota (Boros), Tihany—B. füred, Aszófő—Örvényes, Kapuvár (Soó), Győr, Sárospuszta, Lébény—Sövényháza (Polgár), Zombor (Prodan) — Gyeke (Péterfi), Szamosszentmiklós (Soó, Ujvárosi **var. unicorne**), Gyergyóalfalu (Soó).

III. MYRIOPHYLLUM. **1. M. verticillatum** L. Tiszadada, T. dob—T.-lök (Ujvárosi*), Szamosszeg (Felföldy), — Szigetujfalu (Boros*), Békásmegyer (Lyka), Isaszeg (leg.?*), Ócsa (Soó, Boros pt.**), Dömsöd Boros**), Garamkövesd (Borbás) — Rovnye (Rochel) — Fejér m. Pusztavám, Komárom m. Bokod, Bánhida (Boros), Ölbői tó; Lébény, Győr, Győr-Nádor (*), Gyirmóth und Ikrény ("f. infraflorum"), Pataháza, Kulcsod **(f. pectinatum)**, Börcs (**), Lesenceistvánd-Uzsa major (*-alles Polgár), Kisbalaton (Soó, Walter*), Fenékpuszta (Soó) — Baranya m. Albertfalu (Boros*) — Véke (Margittai) — Kolozsvár (Landoz, Bélteki), Szamosszentmiklós-Apahida (Soó cum*), Kékes (Prodan verg. ad. f. **pectinatum**), Mezőzáh (Prodan), Marosvásárhely (Nyárády**), Balázsfalva (Borza*) Kutyfalva (leg.?), Fenyőfalva (Fuss**), Csíkszentsimon, Cs. csatószeg (Nyárády, sed eius pinnatifidum: typus), Cs. sztkirály (Soó), Tusnádfürdő (Borbás)

2. M. spicatum L. Ohat "Herep" (Soó, Máthé), Nyirmada, Ujfehértó-Érpatak, Hajdubagos (Soó), H, nánás (Soó, Ujvárosi), Kállósemlyén (Péterfi, Soó), Tiszafüred (Máthé), T. dob, T. lök (Ujvárosi), Csap, Örsi (Margittai), Sárospatak, Tapolcafürdő, Szolnok (Soó), Sajókirályi (Boros) Ipolyvece (Noga) — Ungvár, Nevicke (Ubrizsy), — Soroksár (Kocsis, Soó), Ercsi (Tauscher), Kiskunfélegyháza (Boros) — Siófok (Borbás), Koroncó (Ebenhöch), Czétény (Knapp), Győr, Gy. révfalu, Gy. sziget (Polgár), Neszmély, Babocsa (Boros), Kisbalaton (Walter **f. terrestre**) — Jablunka (Jablonszky) — Kolozsvár (Landoz, Bélteki, Wolff, Nyárády, Soó), Oláhbrettye (Páva.i).

*: **f. limosum**, **: **f. pinnatifidum.**

IV. HIPPURIS VULGARIS. L. Vasasszentgothárd-Ördöngösfüzes (Prodan), Koroncó (Ebenhöch), Ács, Bokod, Környe, Komárom m. (Boros)

V. UTRICULARIA **1. U. vulgaris** L. Weitere Standorte aus den Karpathen Kom. Szepes, Trencsén, Szolnok-Doboka und Kolozs. Szepessümeg (Répászky), Skalice (Holuby), Kolozsvár (Landoz, Bélteleki, Borbás, Richter, Nyárády), Mezőőr (Bihari) Vasasszentiván (Győrffy).

3. U. minor L. Tata, Lesenceistvánd (Polgár).

4. U. Bremii Heer Nyirmada (Soó, steril?)

Die Pflanzen des Herb. Kolozsvár unter den Namen: **U. minor** Marosvásárhely leg. Barabás, Kvár 1. Landoz), **U. Bremii** (Városlója leg Knapp) und **U. intermedia** (Légen leg. Wolff) sind alle **U. vulgaris.**

VI. POTAMOGETON.

1. P. natans L. Debrecen "Bedecs" (Soó **ovalifolius**), Kállósemjén "Nagymohos" (Soó **terrestris**), Hajdunánás, Olaszliszka (Ujvárosi **ovalif.**), Királyhelmec "Nagyibolyás" (Margittai F. E. Boh. Sl. 642 ovalif.) — Győr m. Koroncó

(Ebenhöch **vulgaris-ovalif.)**, Garamkövesd (Borbás **vulg., terrestris)**, Nyitra (Knapp **prolixus)**, Pápakovácsi "Attyafürdő" (Boros **vulg., rotundifolius,** verg. ad. **f. amphibius)**, Murabeszterce (Boros **ovalif.)**, Baranya m. Bán (Janka **prolixus)** Kőszeg Alsóerdő (Soó **terrestris)** — Nógrád m. Losonc-Tugár, Miksi (Kunszt **vulg.-ovalif.)** Püspökfürdő (Géczy **prolixus)**, Szilágym. Bogdánvölgy (Soó-Balázs-Felföldy **vulg.-ovalif.)**, Dés (Czetz **vulg.)**, Radnai havasok: Korongyis-Om "Coasta Taului (Boros **vulg., vulg.-ovalif.)**, Naszód (Porcius **vulg.-ovalif.)**, Kolozsvár (Landoz, Wolff **vulg.-ovalif.)** "Szamospart" (Soó **vulg., ovalif., prolixus)**, Szászfenes, Gyalu (Nyárády), Szelicsei tavak (Borbás(?) **terrestris)**, Cege (Prodan **prolixus)**, Noszoly (Prodan **vulg.-ovalif., ovalif.)**, Marosvásárhely (Nyárády **prolixus)**, Csíkszentsimon (Boros **oblongif.)**, Csíkszentkirály, Gyergyóalfalu (Soó **prolixus)**.

2. P. nodosus Poir. Zemplén m. Bodrogzsadány-Bodrogolaszi (Ujvárosi **Billotii)**, Komárom m. Neszmély "Alsósziget" (Boros ad **Billotii** verg.), Baranyavár (Janka **Billotii)** Zala m. Kotor-Alsódomboru (Boros **stagnatilis)**, Váradszöllős-Váradszentmárton (Boros **Billotii)**.

5. P. lucens L. Sárospatak (Hargitai) Tiszalök (Ujvárosi **acuminatus)** Makó "Ludvár" (Halász), Baranya m. Kácsfalu. Dályok-Izsép (Janka **acum.)**, Garamkövesd (Borbás **acum.)**, Koroncó (Ebenhöch), Fonyód-Táska (Lóczy **acum.)**, — Lucski (Borbás **ovalifolius)** — Kolozsvár (Czetz), fragmenta), ex herb. Porc. certe a loco Dés orta), Dés "Horgostó" (Czetz), Nagyenyed (Pávai typus, **ovalif.)**, Cege, Kékes (Prodan), Marosvásárhely (Nyárády).

7. P. gramineus L. Királyhelmec (Margittai F. E. Boh. Sl. 641 **lacustris)**, Fonyód-Táska (Lóczy **stagnalis myriophyllus, fluvialis-stagnalis)**, Szolnok (Soó **stagnalis)**, Tiszaroff (Soó **myrioph.)**, Nagykőrös (Hargitai **lac., stagn.)** — Béllye (Boros **amphibius)**, Rétyi Nyir (Moesz, Ujvárosi **myriophyllus)**.

8. P. perfoliatus L. Budapest „Lágymányos" (Lyka **ovatifolius)**, Tiszalök, Olaszliszka (Ujvárosi **oblongus)** Sárospatak (Hargitai). Nagycétény Nyitra m. (Knapp **cordatolanceolatus)**, Balatonalmádi (Valentini **rotundifolius-densifolius)**, — Kékes (Prodan **cordato-lanceolatus)**.

9. P. crispus L. Tiszadob, Tiszadada-Tiszalök, Hajdunánás (Ujvárosi pt. ad. f. **sinatus** verg.) Hajdubagos, Ujfehértó-Érpatak, Szolnok (Soó), Zombor (Prodan), Baranyavár (Janka), Arad (A. Richter, Szabó) — Losonc, Miksi (Kunszt pt. **planifolius)**, Andornak (leg.?), Rimaszombat (Fábry sub **P. natante)**, Nyitra m. Gajdel (Knapp **major)** — Váradszöllős-Váradszentmárton (Boros), Püspökfürdő (Steffek), Kolozsvár (Landoz, Soó pt. **planifol.)**, Apahida, Gyalu (Soó, Nyárády), Aranyosgyéres (Nyárády), **planif.)**, Gyeke (Péterfi pt. **sinuatus)**, Marosvásárhely (Nyárády), Csíkcsatószeg, Csíkcsikcsó (Nyárády pt. **sinuatus)**, Csíkszentkirály (Soó), Tusnád (A. Richter), Naszód (Porcius).

11. P. acutifolius Link. Baranya m. Dályok-Izsép (Janka), Miksi (Kunszt sub **Zannichellia)**, Tusnádközség (Boros), Rétyi Nyir (Moesz).

13. P. pusillus L. Zala m. Gulács-Gyulakeszi (Soó), Cinkota (Entz M.), Miksi (Fábry, Kunszt), Szigetújfalu (Boros **tenuissimus)**, — Kolozsvár (Landoz sub **Zannichellia**, Soó **major)**, Gyalu, Hidegszamos (Wolff, Soó **major)**, Kisdoboka (Boros **tenuiss.)**, Noszoly (Prodan **vulgaris)**, Marosvásárhely (Nyá-

rády **vulg.**), Radna (Czetz, Porcius **vulg.**), Naszód Porcius **vulg.**), Gyergyóremete (Boros **major**).

14. P. trichoides Cham. et Schlechtd. Királyhelmec (Margittai F. E. Boh. Sl. 640).

15. P. pectinatus L. Debrecen (Soó **tenuifolius**), Csap (Ubrizsy), Tiszalök, Zemplén m. Sára (Ujvárosi **vulgaris**), Sárospatak (Hargitai **scoparius**) Kishortobágy (Hazslinszky **vulg.-scopar.**), Soroksár (Borbás **vulg.-scopar.**), Szeged „Nagyszéksós" (Boros **tenuif.**), Velencei tó: Agárd (Boros **tenuif.-scoparius**, Walger **Vaillantii-scopar.**), Bánhida (Boros **Vaillantii**, pt. **Vaill.-scopar.**), — Neszmély "Alsósziget" (Boros **Vaillantii-balatonicus**), Nagycéteny, Szered (Knapp **vulg.**, pt. **vulg.-Vaillantii**), Tihany-Aszófő "Bozsai öböl" (Felföldy **tenuif.**), Fonyód-Táska (Lóczy **Vaillantii**, **Vaill.-vulg.**), Kisapáti Sztgyörgyhegy (Ujvárosi **Vaillantii**) — Szepesolaszi (Kalchbrenner **scopar.**), Kolozsvár (Landoz sub **P. pusillo**, **vulg.-scopar.**), Szamosfalva (Béltelki sub Zannichellia, **vulg.**), Torda Sósfürdő (Todor F. E. R. 1423 "scoparius": **Vaillantii**. Wolff **vulg.**), Kékes, Noszoly (Prodan **scopar.-Vaill.**), Konca Pávai **scoparius**), Apahida (Ujvárosi **vulg.**)

s.sp. balatonicus (Gams) Soó. Balatonalmádi (Lyka), Velencei tó: Dinnyés, Pákozd-Agárd (Boros). annährend ist die von Bocskay auf der Insel Csepelsziget gesammelte **Vaillantii.**

VIII. **Zannichellia palustris** L. var. **pedicellata** Wahlbg. et Rosén Kolozsvár "Szamospart", Apahida, Szék Soó). Gyulafehérvár (Haynald verg. ad typum), Pata (Nyárády), Radnót, Kutyafalva (Pávai verg. ad typum), — Sivabrada: Zsibra (Kalchbrenner).

Ruppia rostellata Koch. **var. transsilvanica** (Schur) Soó Szamosfalva (Chetianu, Pápai), Torda (Péterfi, Nyárády), Vízakna (Kladni, Fuss, Schur, Römer), Kolozsvár "Melegvölgy" (Soó).

IX. **Najas marina** L.
Morvamező: Magyarfalva (Neilreich), Olaszliszka (Ujvárosi), Cege (Soó)
Najas minor All. Marosvásárhely (Nyárády).

X. **Stratiotes aloides** L. Tiszadob (Ujvárosi).
Hydrocharis morsus-ranae L. Futak (Schneller), Baranyavár (Janka), Kutyfalva Maros-T. m. (Pávai).

XI. **Alisma gramineum** Gmel. Budapest Lágymányos (Lyka **f. angustissimum**), Udvarhely m. Székelyzsombor-Mirkvásár (Nyárády **f. terrestre.**

XII. **Sparganium minimum** Fr. Lucsmelléke Szelicse (Nyárády), Radna (Czetz, Porcius), Tátralomnic "Drei Sechen: Háromtócsa" (Győrffy).

XIII. **Lemna trisulca** L. Görömbölytapolca (Soó), Kolozsvár (Wolff, Bélteki, Knapp), Szamosfalva (A. Richter), Magyarbagó (Pávai), Marosillye (Péterfi), Rétyi Nyir (Moesz).
L. gibba L. Mezőberény (Péterfi), Gáncs (Czetz), Dezmér (Soó).
Spirodela polyrrhiza (L) Schleid. Nagyenyed (Pávai).

XIV. **Calla palustris** L. Bory lápok: Zubrina (Péterfi), Alsószinevér (Ujvárosi), Kosna (Porcius), Déda-Ilva, Kommandó (Walz), Korond: Korondága (Nyárády).

CHROMOSOME NUMBERS OF CERTAIN HUNGARIAN PLANTS.

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From the Hungarian Biological Research Institute Tihany, Lake Balaton.

With 1 Table and 23 figures in the text.

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Cyto-geographical work began in Hungary during the war years. We have at first to make the chromosome-analysis of the Hungarian flora as a basis for further investigations. A study of the literature of this subject is not enough, as investigations along this line have not been sufficient. A complete study of the Hungarian flora from this standpoint is necessary.

I have made studies in the chromosome-numbers of certain plants living in different plant-societies on the peninsula of Tihany, Lake Balaton.

In determining the chromosome numbers I applied the technique which follows: I grew plants in greenhouses, in pots, and fixed the root-tips with Navasin's solution *(Navasin* 1934), and stained them with gentian violet according to Clausen-Johannsen-Oehlkers *(Oehlkers* 1940). In certain cases, especially the buds, I fixed with Carnoy fixative and stained with aceto-carmine *(Geitler* 1940). The degree of magnification is shown by the scale in Figure 1.

In the accompanying Table I give the name and the habitat of the plant, data of literature, and the results of my own investigations.

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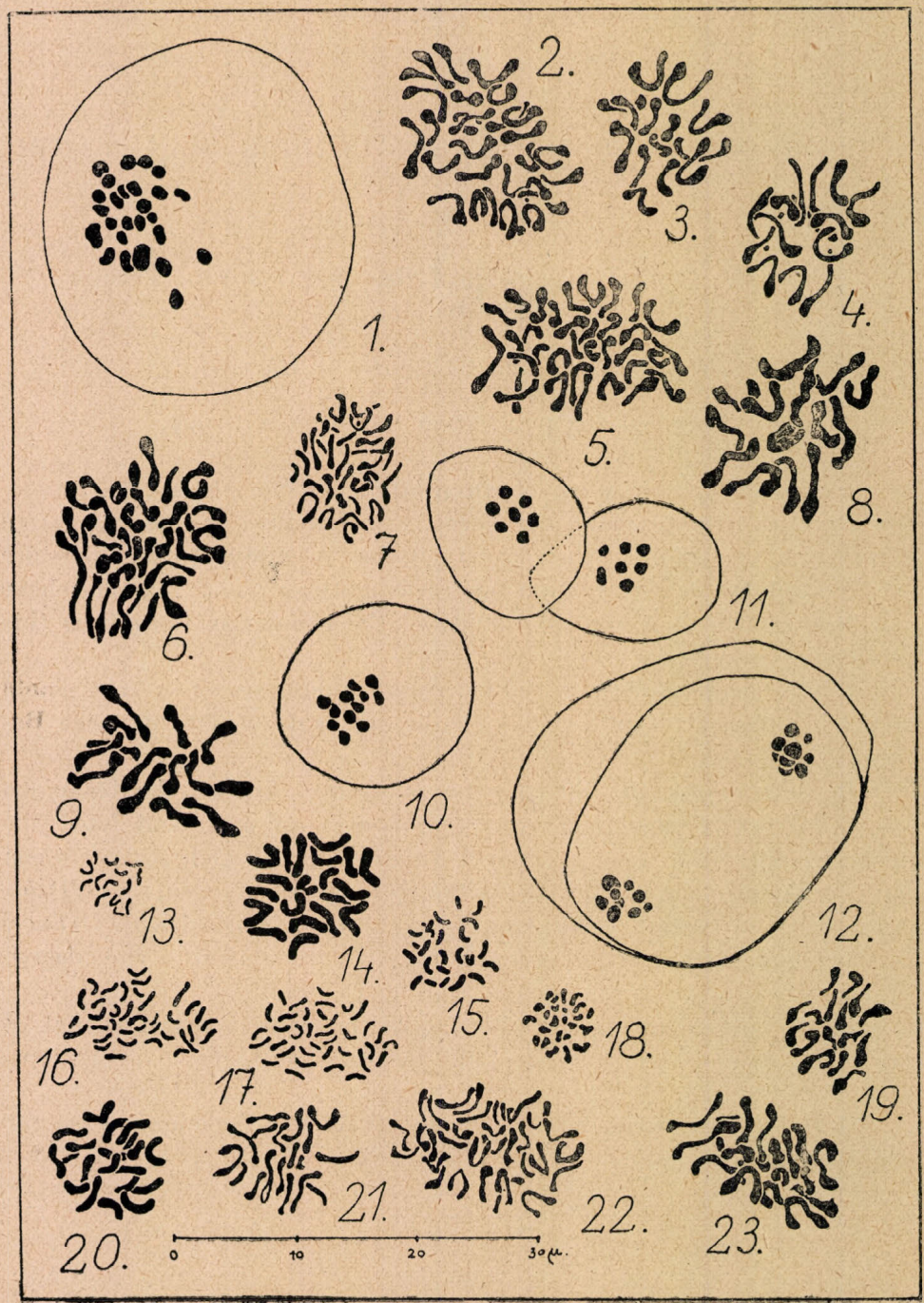
REFERENCES.

Avdulov N. P. 1928: Dnewn. Wcecojusn. Ssesd. Bot. 1. — 1931: Bull. Appl. Bot. Genet. Pl. Breed. Suppl. 43. — Blackburn K. B. 1934: Journ. Hered. 25. Bogyó T. 1941: József N. Müsz. Gazd. Tud. Egyet. Növ. Int. 7. — Bowden W. — Chopinet R.M. 1940: Amer. J. Bot. 27. — 1941: Proc. Va. Acad. Sci. 2.



(Fig. 1.)

TABLE I.

No. Name of plant	Habitat	Data of literature.	Own data.	Methodics.
1. *Potamogeton perfoliatum* L.	Tihany: in lacu Balaton.	n = cca. 24 Wisniewska 1931. n = 26 Palmgren 1939.	n = 26. Fig. 1.	Bud of flower Acetocarmin.
2. *Festuca pseudovina* (Hack.) *Beck.*	Tihany: Akasztódomb.		2n = 28. Fig. 2.	Root-tips. Gentiana.
3. *Bromus commutatus* Schrad.	Tihany: sub monte Óvár.		2n = 14. Fig. 3.	—„—
4. *Bromus tectorum* L.	Tihany: in horto Inst. Biologici.	n = 7 Avdulov 1928, 1931. Stählin 1929. Cugnac-Simonet 1941, -a.	2n = 14. Fig. 4.	—„—
5. *Iris pseudacorus* L.	Tihany: in pratis paludosis Külsőtó.	n = 12 Longley 1928. n = 17 Simonet 1928, 1932. n = 15 Heppel ap. Löve-Löve 1942b.	2n = 34. Fig. 5.	—„—
6. *Caltha palustris* L.	„ „	2n = 58—59 Langlet 1927a. n = 16 Langlet 1927a. n = 16 Levitsky 1931b.	2n = 32. Fig. 6.	—„—
7. *Ranunculus sceleratus* L.	Tihany: in palude Belsőtó.	n = 16 Langlet 1936. Coonen 1939.	2n = 32. Fig. 7.	—„—
8. *R. polyanthemos* L. *f. latifolius* Wallr.	Tihany: in horto Inst. Biologici.	n = 8 Langlet 1932. Larter 1932.	2n = 14. Fig. 8.	—„—
f. villosus Beck.	„ „		2n = 14. Fig. 9.	—„—
9. *Chelidonium majus* L.	Tihany: Templomdomb.	n = 6 Sugiura 1936. Turesson 1938. n = 5 Nagav-Sakai 1939. n = 6 Bowden 1940.	2n = 12. Fig. 10.	Bud of flower Acetocarmin
10. *Papaver rhoeas* L.	Tihany: Kiserdőcsúcs.	n = 7 Ljungdahl 1922. Wilcins et Abele 1927. Lawrence 1930. Jamasaki 1936. Sugiura 1940.	n = 7. Fig. 11.	Bud of flower Gentiana
11. *Sinapis arvensis* L.	Tihany: Templomdomb.	n = 9 Nagai-Sasaoka 1930. Turesson: Löve-Löve 1942. Chopinet 1942.	n = 9. Fig. 12.	Bud of flower Acetocarmin
12. *Fragaria collina* Ehrh. (*Fr. viridis* Duchesne.)	Tihany: sub mt. Akasztódomb.	n = 7 Yarnell 1929a. Rudloff 1930. Schiemann 1937.	2n = 14. Fig. 13.	Root-tips. Gentiana.
13. *Helianthemum ovatum* (Viv.) *f. typicum* Beck. Dun.	Tihany: Óvár.	n = 10 Bowden 1940.	2n = 20. Fig. 14.	—„—
14. *Calystegia sepium* (L.) R. Br.	Tihany: ad ripam lacus Balaton.	n = 11 Kano 1929. n = 12 Percy 1936. Wolcott 1937.	2n = 24. Fig. 15.	—„—
15. *Clechoma hederacea* L. *f. micrantha* (Bönn.) Rouy.	Tihany: sub mt. Akasztódomb.	n = 9 Scheerer (Tischler in litt.) — n = 12 Löve-Löve 1942.	2n = 36. Fig. 16—17.	—„—
16. *Salvia aethiopis* L.	Ad viam versus Tihanyrév.	n = 11 Yakovleva 1933. n = 12 Hruby 1934.	2n = 24. Fig. 18.	—„—
17. *Valeriana dioica* L.	Tihany: Külsőtó.	n = 8 Meurman 1925a., b.	2n = 16. Fig. 19.	—„—
18. *Eupatorium cannabinum* L.	Tihany: ad ripam lacus Balaton.	n = 10 Löve-Löve 1942.	2n = 20. Fig. 20.	—„—
19. *Bellis perennis* L. *f. denticulata* Beck.	Aszófő: ad rivum Aszófői patak.	n = 9 Winge 1917. Blackburn 1934. Negodi 1935, 1937.	2n = 18. Fig. 21.	—„—
20. *Inula conyza* DC.	Tihany: Óvár.	n = 16 Tongiorgi 1935.	2n = 32. Fig. 22.	—„—
21. *Achillea millefolium* L. *var. collina* Becker.	Tihany: Külsőtó.	n = 24 Clausen-Keck-Hiesey 1940; Turesson 1938.	2n = 18. Fig. 23.	—„—

1942: C. R. Acad. Sci. Paris **215.** — Clausen J.—Keck D.—Hiesey W. M. 1940: Carneg. Inst. Wash. Publ. **520.** — Coonen L. P. 1939: Amer. J. Bot. **26.** — Cugnac A.—Simonet M. 1941 a.: Bull. Soc. Bot. France **88.** — 1941: b.: C. R. Soc. Biol. Paris **135.** — Flovik K. 1940: Hereditas (Lund) **26.** — Geitler L. 1940: Schnellmethoden der Kern und Chromosomenuntersuchung Berlin. — Győrffy B. 1939: Acta Biol. Pars. Bot. **5.** — Hagerup O. 1932: Hereditas (Lund) **16.** — 1933: l. c. **18.** — 1938: l. c. **24.** — 1939: l. c. **25.** — 1940: l. c. **26.** — 1941: Bot. Tidskr. **45.** — Hruby K. 1934: Beih. Bot. Cbl. A. **52.** — Jamasaki R. 1936: Jap. Journ. Bot. **8.** — Kanot T. 1929: Proc. Crop. Sci. Soc. Jap. **4.** — Langlet O. 1927: Sv. Bot. Tidskr. **21.** — 1932: l. c. **21.** — 1936: l. c. **30.** — Larter L. N. H. 1932: J. Genet. **26.** — Lawrence W. J. C. 1930: Genetica **13.** — Levitsky G. A. 1931: Bull. appl. Bot. **27.** — Ljungdahl H. 1922: Sv. Bot. Tidskr. **16.** — Longley A. E. 1928: Bull. Am. Iris Soc. **29.** — Löve A.—Löve D. 1942 a.: Kgl. Fysiogr. Lund Förh. **12.** — 1942 b.: Bot. Notiser **1942.** — 1943: Hereditas (Lund) **29.** — Meurman O. 1925: Soc. Sci. Fenn., Comn. Biol. **2.** — 1925 b.: l. c. — Nagai K.—Sasaoka T. 1930: Jap. J. Genet. **5.** — Nagao S.—Sakai K. 1939: Jap. J. Genet **15.** — Navasin M. 1934: Zeitschr. f. Züchtg. A. Pflanzenzüchtg. **19.** — Negodi G. 1935: Atti Soc. Nat. Mat. Modena **67.** — 1936: Biol. general. **12.** — 1937: l. c. — Nicholson C. 1939: Rev. Univ. Arequipa **12.** No. **14.** — Oehlkers F. 1940: Z. Bot. **36.** — Palmgren O. 1939: Bot. Notiser **1939.** — Persy I. 1936: Bull. Soc. Roy. Belg. **68.** — Rohweder H. 1936: Beih. Bot. Cbl. A. **54.** — Rudloff 1930: Gartenbauwiss. **3.** — Scheerer (Tischl. in litt) ap. Löve-Löve 1942. b. — Schiemann E. 1937: Zeitschr. ind. Abst. Vererb. 73. — Simonet M. 1928: C. R. Soc. Biol., Paris **99.** — 1932: These, Paris **1932.** — Sokolovskaja A. P.—Strelkova O. S. 1941: C. R. (Doklady) Acad. Sci. URSS. **32.** — Stählin A. 1929: Wiss. Arch. Landwirtsch. **1.** — Sugiura T. 1936: Cytologia (Tokyo) **7.** — 1940: l. c. **10.** — Tischler G. 1931: Tab. Biol. **VII.** — 1935—36: l. c. **XI—XII.** — 1938: l. c. **XVI.** — 1937: Cytologia (Tokyo) **8.** — Tongiorgi E. 1935: N. Giorn. Bot. Ital. **42.** — Turesson G. 1938: Ann. Agric. Coll. Sweden **5.** — Wilkins H.—Abele N. 1927: Acta Hort. Bot. Latviensis **2.** — Winge O. 1917: C. R. Carlsb. **13.** — Wisniewska N. 1931: Acta Soc. Bot. Polon. **8.** — Wolcott G. B. 1937: Amer Nat. **71.** — Wulff H. D. 1938: Ber. deutsch. Bot. Ges. **56.** — 1939: l. c. **57.** — Jakovleva S. V. 1933: Bull. appl. Bot. Ser. 2. Nr. **5.** — Yarnell S. H. 1929: Genetics **14.** —

Fig. 1. Potamogeton perfoliatum $n = 26$; 2. Festuca pseudovina $2n = 28$; 3. Bromus commutatus $2n = 14$; 4. B. tectorum $2n = 14$; 5. Iris pseudacorus $2n = 34$; 6. Caltha palustris $2n = 32$; 7. Ranunculus sceleratus $2n = 32$; 8. R. polyanthemos f. latifolius $2n = 14$; 9. R. polyanthemos f. villosus $2n = 14$; 10. Chelidonium majus $2n = 12$; 11. Papaver rhoeas $n = 7$; 12. Sinapis arvensis $n = 9$; 13. Fragaria collina $2n = 14$; 14. Helianthemum ovatum $2n = 20$; 15. Calystegia sepium $2n = 24$; 16—17. Glechoma hederacea f. micrantha $2n = 36$; 18. Salvia aethiopis $2n = 24$; 19. Valeriana dioica $2n = 16$; 20. Eupatorium cannabinum $2n = 20$; 21. Bellis perennis $2n = 18$; 22. Inula conyza $2n = 32$; 23. Achillea millefolium var. collina $2n = 18$.

SOZIOLOGISCH-CYTOGEOGRAPHISCHE UNTERSUCHUNGEN ÜBER DIE PANNONISCHE RUDERALVEGETATION.

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(Mit 2 Abbildungen und 2 Tabellen im Text.)

(Eingegangen am 1. November 1946.)

I. Einleitung.

Bei der cytogeographischen Untersuchung der ungarischen Flora wandte ich besondere Sorgfalt für die auch vom praktischen Gesichtspunkte aus wichtigen Ruderalpflanzen auf. Ich beabsichtige die cytologischen Ergebnisse späterhin zu veröffentlichen, in vorliegender Arbeit berichte ich nebst einer kurzen soziologischen Besprechung über die cytogeographische Analyse der Pflanzengesellschaften. Diese Analyse führte ich nicht allein auf Grund schrifttümlicher Angaben durch (Tischlers Tabellen 1927—39 u. a.), benutzte vielmehr auch meine eigenen Untersuchungen mit dem Ergebnisse, dass ich den Prozentwert der unbekannten Pflanzen in den einzelnen Assoziationen auf 0—11% herabzudrücken vermochte.

Die soziologischen Resultate habe ich 1942 z. T. veröffentlicht (Felföldy, 1942, p. 87); seit damals habe ich mehrere solche soziologischen Aufnahmen gemacht, die zusammen mit den alten in vorliegender Arbeit verwertet wurden.

Meine Aufnahmen stammen aus der Klimaxzone der *Quercion roboris* der pannonischen Florenprovinz *(Pannonicum)* aus den Bezirken des ung. Mittelgebirges *(Matricum)*, des Alfölds *(Eupannonicum)* und des *Transdanubicums* (vgl. Soó 1940, p. 19 und die Karte auf Taf. I). In der Region der Buchenwälder habe ich keine Untersuchungen durchgeführt.

II. Soziologischer Teil.

Die Prinzipien der Systematik, Nomenklatur und Aufnahme der Pflanzenvereine finden sich samt der kritischen Behandlung der Schrifttumsangaben in meiner erwähnten Arbeit. Unsere Ruderalgesell-

schaften wurden nach den physiognomischen, ökologischen und floristischen Unterschieden wie folgt eingeteilt:

1. a. Therophyten dominierend — — — — — — — 2
1. b. Hemikryptophyten dominierend — — — — — 3
2 a. Hygrophile Vereine niedrigen Wuchses, physiognomisch und floristisch mit der Schlammvegetation nahe verwandt, doch menschlich beeinflusst: Emersiherbosa: *Nanocyperion.*
2. b. Meso- oder xerophile Vereine, wenn hygrophil, dann mit doppelter Feldschicht (Physiognomie der Hochstaudenfluren bzw. Lagerfluren). Arvideserta — 4
3. a. Wiesenartige Vereine: Sempervirentiherbosa: *Lolion.*
3 b. Gebüsche Aestifruticeta: *Prunion spinosae.*
4 a. Hohe Pflanzengesellschaften mit 2 Feldschichten 5
4 b. Niedrige „ „ 1 Feldschicht 7
5 a. Hygrophile Vereine — — — *Polygono-Chenopodion.*
5 b. Meso- oder xerophile Vereine (mit viel Zweijährigen: Hemitherophyten: TH) — — — — — — — — 6
6 a. Stark gedüngter, nicht betretener, mehr mesophiler Standort, Gesellschaften ohne Stechpflanzen: *Arction lappae.*
6 b. Wenig gedüngter (weniger nitrophiler!), betretener, oft beweideter, mehr xerophiler Standort, Stechplanzenvereine — — — — — — *Onopordion acanthii.*
7 a. An betretenen Stellen, Wegen, Wegrändern meist Vereine von niedrigem Wuchs — — — *Polygonion avicularis.*
7 b. An gestörten, aber kaum betretenen Standorten, Vereine oft mit höherem Wuchs, jedoch mit einer Feldschicht *Secalinion medioeuropaeum.*

Die Assoziationen sind im einzelnen die folgenden:

A. Emersiherbosa — *Sumpfpflanzengesellschaften.*

I. Nanocyperion flavescentis Koch 1926.

1. Juncus bufonius-Ass. Dieser sehr verbreitete Zwergbinsenverein ist im Gegensatze zu den bisher bekannten schlammbewohnenden Rasen vom *Nanocyperion*-Typus als morastbewohnende Assoziation anzusprechen. Er kommt an unter kulturellen Wirkungen stehenden Stellen vor: auf schlammigen Stoppelfeldern, feuchten, betretenen und gedüngten Gänseweiden sowie von Tieren betretenem, gewissermassen gekne-

tetem Schlamme. Ich habe 2 Variationen studiert: a. Juncus *bufonius-Echinochloa crus-galli Subass.*, die sich in den Vertiefungen sumpfiger Stoppelfelder und Ackerwege vorfindet und *b. Juncus bufonius Potentilla anserina Subass.*, die einen typischen Verein gedüngter Gänseweiden darstellt (vgl. S o ó 1940, p. 42).

1. a. *Juncus bufonius-Echinochloa crus-galli Subass.* Charakteristische Artenkombination:* K = 5: *Echinochloa crus-galli, Juncus bufonius, Lythrum hyssopifolia, Anagallis arvensis, Centaurium pulchellum, Kickxia elatine, Veronica anagalloides, Plantago major ssp. intermedia;* K = 4: *Polygonum persicaria, Chenopodium album, Potentilla supina, Convolvulus arvensis, Sonchus asper;* K = 3: *Lolium perenne, Polygonum aviculare, Arenaria serpyllifolia, Consolida regalis, Ranunculus sardous, Medicago lupulina, Ajuga chamaepithys, Erigeron canadense, Taraxacum officinale, Sonchus arvensis.* — Wichtige Arten: *Cyperus fuscus* (A—D:1, K:1), *Verbena officinalis* (1,2), *Kickxia spuria* (1, 1), *Gnaphalium luteo-album* (1, 1), *Aethusa cynapium* (1, 1).

Artenzahl: 45.

Konstitutionsspektrum: K5: 17, 7; K4: 11, 1; K3: 22, 2; K2: 24, 5; K1: 24, 5.

Bio-ökologisches Spektrum: Th: 73, 4; H: 20; TH: 2, 2; G: 4, 4%.

Verteilung der Elemente: M: 15, 6; K: 24, 5; Adv: 6, 6; Cp: 6, 6; Eua: 35, 5; Eu: 11, 1%.

Cytologische Daten: d: 62, 2; P: 29, 8; i: 8, 0%; d/P: 2, 08; (P)%: 30%.

1 b. *Juncus bufonius Potentilla anserina-Subass.* Charakteristische Artenkombination: K = 5: *Poa annua, Juncus bufonius, Chenopodium glaucum, Spergularia rubra, Potentilla anserina, P. supina, Lotus corniculatus, Malva neglecta (incl. pusilla), Lythrum hyssopifolia, Anagallis arvensis, Centaurium pulchellum, Verbena officinalis, Plantago major p. p. ssp. intermedia, Pulicaria vulgaris, Matricaria inodora;* K = 4: *Trifoluim fragiferum;* K = 3: *Rumex sarguineus, Erigeron canadense, Bidens tripartita.*

Wichtige Arten: *Veronica anagalloides* (1, 1), *Pulicaria dysenterica* (1, 1).

Artenzahl: 26.

Konstitutionsspektrum: K5: 57, 7; K4: 3, 8; K3: 11, 6; K2: —; K1: 26, 9%.

Bio-ökologisches Spektrum: Th: 61, 5; H: 34, 6; TH: 3, 9%.

* Erklärung der Abkürzungen und der Methodik s. S. 128.

Verteilung der Elemente K: 30, 7; Adv: 7, 7; Cp: 3, 9; Eua: 50, 0; Eu: 7, 7%.

Cytologische Daten: d: 67, 8; P: 43, 1; i: 1, 0%; d/P: 1, 38; (P)%: 40%.

(Vgl. Soó 1940, p. 42, Polgár 1912, p. 320, Zólyomi 1937, p. 181, Moor 1936, p. 119.)

B. Sempervirentiherbosa — *Immergrüne Wiesen.*

II. Lolion perennis Felföldy 1942.

Diejenigen immergrünen Ruderalien, in deren ökologischem Spektrum die einjährigen Elemente die 50% nicht erreichen, die zweijährigen Pflanzen keine bedeutende Rolle spielen und nur eine einfache Feldschicht aufweisen, sind in den *Lolion-Verband* einzureihen. Sie bilden nicht allein vom physiognomischen, vielmehr auch vom physiognomisch-biologischen Gesichtspunkte aus einen Übergang von den Vereinen vom Typus der ephemeren *Arvideserta* zu denen der natürlichen *Sempervirentiherbosa*. Hierhin gehören hauptsächlich mesophile, in geringerem Masse hygrophile Assoziationen *(Potentilla anserina*-Ass.), die das Betreten (vor allem *Lolietum),* Düngen *(Potentilletum)* und Bodenstörungen *(Agropyretum)* gut vertragen. Unter ihnen ist die *Cynodon dactylon-Ass.* am anspruchsvollsten, abwechslungsreichsten, dagegen komm sie bereits auf natürlicheren Standorten vor.

2. *Agropyron repens-Ass* Diese ist eine Pioniergesellschaft an Plätzen, wo die natürlichen Verhältnisse durch irgendeine Wirkung eine Störung erlitten haben und die Pflanzenwurzeln zerstört worden sind. Das zähe, verqueckte *Agropyron* indessen lebt weiter, denn jedes Stückchen der zerkleinerten Stolo treibt wieder. Häufig findet sich dieser Pflanzenverein auf Bahnböschungen, entlang von Gräben, inmitten von Anpflanzungen, doch kommt er auch an den Rändern natürlicher Risse, Schutte vor. An Ufern, Dämmen wird er durch das Vorwiegen von Calystegia sepium charakterisiert (Felföldy 1943a, p. 55), an seinen wirklich ruderalen Standorten durch das von Convolvulus arvensis. Nachstehend führe ich diesen vor.

Charakteristische Artenkombination: K = 5: *Agropyron repens, Polygonum aviculare, Convolvulus arvensis;* K = 4: *Plantago lanceolata, Artemisia vulgaris;* K = 3: *Erigeron canadense.*

Wichtige Arten: *Lolium perenne, Berteroa incana, Ballota nigra, Achillea collina,* (A—D: 1—2, K: 2).

Artenzahl: 55.

Konstitutionsspektrum: K5: 5, 5; K4: 5, 5; K3: 1, 8; K2: 20; K1: 67, 2%.

Bio-ökologisches Spektrum: Th: 49, 1; H: 29, 1; TH: 14, 5; G: 7, 3%.

Verteilung der Elemente: Kont: 1, 8; Pm: 1, 8; M: 14, 5; K: 14, 5; Adv.: 1, 8; Cp: 11, 0; Eua: 36, 4; Eu: 9, 1; Em: 9, 1%.

Cytologische Daten: d: 66, 6; P: 26, 3; i: 6, 6%; d/P: 2, 54; (P)%: 30%.

(Vgl. Gams 1927 Agropyron intermed.-Ass. p. 221.)

3. *Lolium perenne-Ass.* Die verbreitetste immergrüne Ruderalie. Sie ist in den Vorstädten, Parks und Gärten von Berlin, Budapest und Debrecen ebenso auffindbar wie auf den sodahaltigen Weiden der Hortobágy oder in den Wagenspuren der Ackerwege des transdanubischen Ackerlandes. In Nachstehendem befasse ich mich lediglich mit den Lolieten der ausgesprochen ruderalen Standorte, nicht aber mit denen der natürlicheren Fundstellen (Wiesen, Rodeland usw.).

Charakteristische Artenkombination: K5: *Lolium perenne, Polygonum aviculare, Erigerom canadense;* K4: *Chenopodium album, Trifolium repens, Plantago lanceolata, Taraxacum officinale;* K3: *Setaria glauca, Apera spica-venti, Dactylis glomerata, Poa annua, Hordeum murinum, Plantago major, Achillea millefolium resp. collina.*

Artenzahl: 67.

Konstitutionsspektrum: K5: 4, 3; K4: 5, 8; K3: 10, 1; K2: 11, 6; K1: 68, 2%.

Bio-ökologisches Spektrum: Th: 44, 9; H: 34, 8; TH: 13, 0; G: 7, 3%.

Verteilung der Elemente: Kont: 2, 9; P: 1, 4; M: 5, 8; K: 20, 3; Adv: 7, 3; Cp: 10, 1; Eua: 42, 0; Eu: 4, 3; Em: 5, 8 %.

Cytologische Daten: d: 62, 4%; P: 33, 4; i: 4, 2%; d/P: 1, 86; (P)%: 31, 1%.

(Vgl. Gams 1927, p. 380, Beger 1930, p. 511, Soó 1932, p. 118, 1933, p. 15, 1936, p. 356, Aichinger 1933, p. 61, Tüxen 1937, p. 22, Pfeiffer 1937, p. 599, Pénzes 1942, p. 84 und 118, Felföldy 1942, p. 104 usw).

4. *Potentilla anserina-Ass.* Man pflegt diese Pflanzengesellschaft als Charakteristikum der Gänseweiden zu erwähnen, doch hat sie eine weitaus grössere Verbreitung. Tatsächlich ist sie sehr häufig auf den feuchteren Stellen der Gänseweiden der transdanubischen Dörfer, und dann wird sie vom Vorhandensein von *Trifolium fragiferum* gekenn-

zeichnet, doch ist sie auch auffindbar in flacheren Gräben, auf den Fusswegen von Wiesen, auf Ackerwegen, an den Rändern von Ackerland.

Charakteristische Artenkombination: K5: *Lolium perenne, Potentilla anserina, Lotus corniculatus, Plantago major;* K4: *Poa annua, Trifolium fragiferum, Plantago lanceolata, Achillea millefolium;* K3: *Polygonum aviculare, Medicago lupulina, Centaurium pulchellum, Odontites rubra.* — Wichtige Arten: *Juncus bufonius* (A—D: +—1, K:2), *Trifolium repens, Prunella vulgaris, Matricaria inodora* (1, 1).

Artenzahl: 60.

Konstitutionsspektrum: K5: 6, 7; K4: 6, 7; K3: 6, 7; K2: 11, 6; K1: 68, 3%.

Bio-ökologisches Spektrum: Th: 38, 4; H: 46, 6; TH: 10, 0; G: 5, 0%.

Verteilung der Elemente: P: 1, 6; M: 3, 4; K: 23, 3; Adv: 1, 6; Cp: 1, 6; Eua: 50, 0; Eu: 13, 4; Em: 5, 0%.

Cytologische Daten: d: 51, 9; P: 46, 8; i: 1, 3%; d/P: 1, 11; (P)%: 44, 8%.

(Vgl. Soó 1940, p. 42.)

5. *Cynodon dactylon-Ass.* Der Rasen des *Cynodon dactylon* ist ausserordentlich dynamisch und unterdrückt vermittels der Sukzession die *Lolieten* aus dem Grunde nicht, weil er anspruchsvoller als diese ist. Er ist nicht allein auf ruderalen Standorten, wie Strassenrändern, Grabenrändern, Eisenbahndämmen, vielmehr auch auf natürlicheren Weiden, trockeneren Wiesen verbreitet.

Charakteristische Artenkombination: K5: *Cynodon dactylon;* K4: *Polygonum aviculare, Chenopodium album, Melandrium album, Erigeron canadense;* K3: *Apera spica-venti, Poa pratensis incl. angustifolia, Lolium perenne, Agropyron repens, Hordeum murinum, Berteroa incana, Convolvulus arvensis, Anchusa officinalis, Plantago lanceolata, Achillea collina, Cichorium intybus, Taraxacum officinale.* — Wichtige Arten: *Equisetum ramosissimum, Calamagrostis epigeios, Plantago indica* usw.

Artenzahl: 70.

Konstitutionsspektrum: K5: 1, 4; K4: 5, 5; K3: 16, 6; K2: 4, 2; K1: 72, 2%.

Bio-ökologisches Spektrum: Th: 36, 1; H: 34, 7; TH: 16, 6; G: 8, 4; Ch: 2, 8; Ph: 1, 4%.

Verteilung der Elemente: Kont: 5, 5; M: 1, 4; K: 19, 5; Adv: 6, 9; Cp: 6, 9; Eua: 48, 7; Eu: 5, 5; Em: 5, 5%.

Cytologische Daten: d: 72, 1: P: 21, 8; i: 6, 1%; d/P: 3, 30; (P)%: 27, 9%.

(Vgl. Gams 1927, p. 380, Soó 1932, p. 117, 1940, p. 42, Magyar 1933, p. 300, Bojko 1933, p. 207, Hargitai 1940, p. 212.)

C. Aestifruticeta — *Gebüschgesellschaften.*

III. Prunion spinosae Soó 1930—31.

Die typischste Eigenheit der Gebüschruderalien im Vergleich mit krautigen Unkräutern ist deren grössere Beständigkeit, und in der Tat sind sie die dem Klimax am nächsten stehenden Pflanzenvereine.

6. *Sambucus ebulus-Ass.* Ich musste die *Sambucus ebulus-Ass.* diese weitverbreitete, typische Assoziation, entgegen ihrer hemikryptophytischen Beschaffenheit in das die Gebüschgesellschaften in sich schliessende Prunion einreihen. Eigentlich ist sie ein Übergang zu *Artion lappae,* doch von ausgesprochenem *Prunion typus.* In ihrem ökologischen Spektrum ist der Th-Wert unter allen untersuchten Vereinen der niedrigste (32, 8%); auch ihre Physiognomie weist mehr auf Gebüschgesellschaften hin; ihre Standorte und Ansprüche aber erinnern vollkommen an die Mitglieder des Prunionverbandes. Sie findet sich abwechselnd mit *Lycium-* oder *Prunus*-gebüschen und ist weitaus beständiger als die *Arction*-Assoziationen.

Charakteristische Artenkombination: K5: *Lolium perenne, Polygonum aviculare, Melandrium album, Ballota nigra, Sambucus ebulus, Artemisia vulgaris;* K4: *Cannabis sativa, Chenopodium album, Torilis anthriscus, Convolvulus arvensis, Erigeron canadense, Cichorium intybus;* K3: *Agropyron repens, Urtica dioica, Achillea millefolium, Carduus acanthoides.* A—D = 3: *Clematis vitalba.*

Artenzahl: 64.

Konstitutionsspektrum: K5: 9, 4; K4: 9, 4; K3: 6, 2; K2: 10, 9; K1: 64, 1%.

Bio-ökologisches Spektrum: Th: 32, 8; H: 32, 8; TH: 23, 4; G: 6, 2; Ch: 1, 5; Ph: 3, 1%.

Elementenverteilung: Kont: 1, 5; P: 1, 5; Pm: 3, 1; M: 4, 8; K: 14, 3; Adv: 6, 2; Cp: 10, 9; Eua: 45, 3; Eu: 6, 2; Em: 6, 2%.

Cytologische Daten: d: 69, 9; P: 20, 2; i: 9, 9%; d/P: 3, 42; (P)%: 29, 8%.

7. *Prunus spinosa-Ass.* Die Schlehengebüsche sind in Ungarn sehr verbreitet. Sie sind wichtig als Strauchschicht der Steppenwälder, als Stachelvegetation der mit Gesträuch bewachsenen Weiden (*Pruneto-Crataegetum* Soó 1931, p. 312, 1940, p. 38), schliesslich als wirklich

ruderale Sträucher von Hecken entlang der Strassen und Wege (*Prunus spinosa-Ballota nigra-Ass.* s. u.)

Charakteristische Artenkombination: K5: *Melandrium album, Prunus spinosa, Ballota nigra, Erigeron canadense, Artemisia vulgaris;* K4: *Cannabis sativa, Polygonum aviculare, Chenopodium album, Atriplex tatarica, Berteroa incana, Lactuca serriola;* K3: *Hordeum murinum, Fagopyrum dumetorum, Atriplex oblongifolia, Torilis anthriscus, Convolvulus arvensis, Carduus acanthoides.* — Wichtige Arten: *Crataegus monogyna, Euonymus europaea.*

Artenzahl: 43.

Konstitutionsspektrum: K5: 11, 7; K4: 14, 0; K3: 11, 6; K2: 16, 3; K1: 46, 4%.

Bio-ökologisches Spektrum: Th: 41, 8; H: 34, 9; TH: 16, 2; G: 4, 7; Ph: 2, 4%.

Elementenverteilung: Kont: 4, 7; Pm: 4, 7; M: 2, 4; K: 14, 0; Adv: 6, 7; Cp: 9, 3; Eua: 46, 5; Eu: 9, 3; Em: 2, 4%.

Cytologische Daten: d: 67, 4; P: 23, 3; i: 9, 3%; d/P: 2, 88; (P)%: 42, 1%.

(Vgl. Soó 1931, p. 312, 1940. p. 38; Felföldy 1942, p. 110).

8. *Lycium halimifolium-Ass.* Ein mit den beiden vorhergehenden abwechselnd wachsender, doch der typischste Heckenverein ist das Lycetum. Es ist eine echte Ruderalie, nur in der Nähe menschlicher Siedlungen zu finden, worauf auch ihre vielen (12, 5%) adventiven Elemente hinweisen. Auch das Lycium selbst ist eine eingeschleppte fremde Pflanze.

Charakteristische Artenkombination: K5: *Polygonum aviculare, Melandrium album, Ballota nigra, Lycium halimifolium, Erigeron canadense;* K4: *Lolium perenne, Agropyron repens, Torilis anthriscus;* K3: *Hordeum murinum, Cannabis sativa, Robinia pseudacacia, Carduus acanthoides.*

Artenzahl: 68.

Konstitutionsspektrum: K5: 6, 9; K4: 4, 2; K3: 5, 5; K2: 20, 8; K1: 62, 5%.

Bio-ökologisches Spektrum: Th: 43, 0; H: 25, 0; TH: 22, 3; G: 5, 5; Ph: 4, 2%.

Verteilung der Floraelemente: Kont: 2, 8; M: 2, 8; K: 16, 6; Adv: 12, 5; Cp: 6, 9; Eua: 40, 2; Eu: 9, 7; Em: 8, 4%.

Cytologische Daten: d: 69, 0; P: 25, 5; i: 5, 5%; d/P: 2, 70; (P)%: 32, 8%.

(Vgl. Felföldy 1942, p. 111, 1943a. p. 118).

D. Arvideserta — *Kulturgeseilschaften.*

Bidention tripartitae *Nordhagen 1940.*

Sie sind hygrophile Ruderalien. Pfützen, die Gräben morastiger Dorfränder dienen ihnen ebenso als Wohnsitze wie die Ufer grösserer Flüsse, Seen, wo sie ihren Stickstoffbedarf aus den verfaulenden Sumpfpflanzen decken (Aichinger 1933, p. 54). Im Gegensatz zu den westlichen Verfassern sind bei uns keine hierhin gehörenden Pflanzenvereine bekannt, die keinen solchen völlig feuchten Standort hätten, was in Anbetracht unseres kontinentalen Klimas auch verständlich ist. In den westlicher von uns liegenden Gegenden lebt selbst auf den feuchteren Stoppelfeldern eine Vegetation vom Typus Bidention. Von wirklich ruderalem Standort kennen wir nur die Bidenteten; die *Polygonum lapathifolium-Ass.* ist bereits Bewohner natürlicherer Standorte, wie Sandbänke, Schurfstellen (vgl. Zólyomi 1937, p. 181, Ujvárosi 1940, p. 32, Felföldy 1942, p. 115, 1943a, p. 63, 1943 b, p. 119).

9. *Bidens tripartita-Ass.* Sämtliche Verfasser stimmen darin überein, dass das Bidentetum eine nitrophile und feuchtigkeitsliebende Assoziation ist. Der ganze Verband verdient die weitere Erforschung.

Charakteristische Artenkombination: K5: *Bidens tripartita;* K4: *Rumex sanguineus, Polygonum lapathifolium, Potentilla anserina, Plantago major, Erigeron canadense, Matricaria inodora;* K3: *Polygonum aviculare, Chenopodium album, Malva neglecta.* — Wichtige Arten: *Chenopodium polyspermum, Ch. glaucum, Ranunculus sceleratus, Dipsacus silvester, Pulicaria vulgaris, Mentha longifolia Alopecurus geniculatus* usw.

Artenzahl: 86.

Konstitutionsspektrum: K5: 1, 1; K4: 6, 9; K3: 3, 5; K2: 16, 0; K1: 72, 4%.

Bio-ökologisches Spektrum: Th: 49, 4; H: 34, 5; TH: 10, 4; G: 3, 5; Ch: 1, 1; HH: 1, 1 %.

Verteilung der Florenelemente: Kont 1, 1; P: 1, 1; M:2, 3; K: 19, 6; Adv: 9, 2; Cp: 5, 7; Eua: 44, 8; Eu: 10, 4; Em: 4, 6%.

Cytologische Daten: d: 51, 7; P: 46, 9; i: 1, 4%; d/P: 1, 10; (P)%: 45, 0%.

(Vgl. Allorge 1922, p. 115, Koch 1926, p. 28, 1931, p. 107, Libbert 1930, p. 34 1932, p. 36. Aichinger 1933, p. 54, Schwik-

kerath 1933, p. 124, Klika 1935, p. 119, Braun-Blanquet 1936, p. 8., Tüxen 1937, p. 26, Bartsch 1940, p. 31, Soó 1932, p. 119, 1934, p. 706, Nordhagen 1940, p. 8. Felföldy 1942 p. 113, 1943 a, p. 63, 1943 b, p. 119, Morariu 1943, p. 177. Klika 1944 p. 21.)

V. Arction lappae Tüxen 1937.

In diesen Verband gehören von den typischen Vereinen der wirklichen Ruderalien die mistbewohnenden, nitrophilen, das Betreten und die mechanischen Wirkungen wenig vertragenden hochstaudigen Gesellschaften. Hierhin zähle ich von den ungarländischen mesophilen Ruderalien diejenigen, die sonst in den *Chenopodion muralis*-Verband gehören würden (Aichinger 1933, p. 56). Gestützt auf meine bisherigen Forschungen fand ich keinen Grund, sie vom *Arction* abzutrennen.

10. *Malva neglecta-Ass.* Eine wirkliche ruderale Gesellschaft, wie jedes Mitglied des Arctions. Sie entwickelt sich ausschliesslich in unmittelbarer Nähe menschlicher Siedlungen, auf stark gedüngten Stellen, neben Dünger- und Misthaufen, Wegen, in trockenen Gräben, an den trockensten Stellen von Gänseweiden. Eigentlich gehören hierhin die unter dem Namen *Urtica-Chenopodium* erwähnten, um Häusern herum stehenden Ruderalien (Soó 1940, p. 42), die nur an niederschlagsreicheren Örtern in typischer Weise sich entfalten können.

Charakteristische Artenkombination: K5: *Polygonum aviculare, Chenopodium glaucum, Malva neglecta;* K4: —; K3: *Chenopodium album, Atriplex tatarica, Amaranthus retroflexus, Verbena officinalis, Erigeron canadense, Anthemis arvensis.* — Wichtige Arten: *Urtica urens, U. dioica, Chenopodium urbicum, Ch. murale, Mercurialis annua, Malva pusilla, Solanum nigrum, S. villosum, Matricaria inodora, Hyoscyamus niger, Datura stramonium* usw.

Artenzahl: 61.

Konstitutionsspektrum: K5: 5, 0; K4: —; K3: 10, 0; K2: 28, 3; K1: 56, 7%.

Bio-ökologisches Spektrum: Th: 53, 4; H: 35, 0; TH: 10, 0, G: 1, 6%.

Verteilung der Floreneiemente: Kont: 1, 6; M: 1, 6; K: 21, 6; Adv: 10, 0; Cp: 3, 4; Eua: 45, 0; Eu: 13, 4; Em: 3, 4%.

Cytologische Daten: d: 55, 1; P: 35, 7; i: 9, 2%; d/P: 1, 54; (P)%: 36, 3%.

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(Vgl. Aichinger 1933, p. 56 *Hyoscyamus niger- Malva neglecta* Ass.; Felföldy 1942, p. 116, Morariu 1493, p. 156 *Malvetum pusillae).*

11. *Solanum nigrum-Ass.* Typischer Verein feuchter, mistiger Stellen, besonders aber auf Kompostlagern verbreitet. Die grosse Anzahl der Th-Elemente weist auf ein sehr karges Biotop hin, und es ist interessant festzustellen, dass diese Gesellschaft an Örtern mit intensiver Bodenstörung wächst, wo also die Sukzession stets von neuem beginnt.

Charakteristische Artenkombination: K5: *Digitaria sanguinalis, Urtica dioica, Chenopodium album, Amaranthus retroflexus, Solanum nigrum, Galinsoga parviflora;* K4: *Setaria glauca, Chenopodium hybridum, Amarathus adscendens, Stellaria media;* K3: *Echinochloa crus-galli, Setaria verticillata, Urtica urens, Amaranthus albus, Melandrium album, Erigeron canadense.* — Wichtige Arten: *Portulca oleracea, Chelidonium majus, Malva neglecta, Hibiscus trionum, Xanthium strumarium, Datura stramonium;* verwilderte Kulturpflanzen: *Solanum lycopersicum, S. tuberosum, Cucurbita pepo, Calendula officinalis, Solidago canadensis, Helianthus annuus, Anethum graveolens* usw.

Artenzahl: 72.

Konstitutionsspektrum: K5: 6, 9; K4: 8, 4; K3: 6, 9; K2: 19, 4; K1: 58, 4%.

Bio-ökologisches Spektrum: Th: 62, 5; H: 23, 6; TH: 4, 2; G: 4, 2; M: 5, 5%.

Verteilung der Florenelemente: Kont: 1, 4; M: 1, 4; K: 22, 3; Adv: 26, 4; Cp: 4, 1; Eua: 31, 9; Eu: 11, 1; Em: 1, 4%.

Cytologische Daten: d: 39, 1; P: 47, 9; i: 13, 0%; d/P: 0, 82; (P)%: 50%.

(Vgl. Pfeiffer 1940, p. 124: *Papaver somniferum — Solanum lycopersicum-Ass.* von Komposthaufen.

12. *Arctium lappa-Ass.* Die typischste Arctiongesellschaft. Sie kommt an verschiedenen Standörtern auf Strassen, Höfen, Misthaufen, sogar Ackerfeldern vor. Die übrigens zweijährigen *Arctium*-Arten blühen bei uns meistens bereits im ersten Jahre und gehen auch zugrunde. Im Alföld bildet *Arctium lappa* Bestände, in Transdanubien und in der Umgebung des Balatons wird es oft durch. *A. tomentosum* vertreten; in Tihany habe ich auch die Dominanz von *A. minus* beobachtet.

Charakteristische Artenkombination: K5: *Polygonum aviculare, Chenopodium album, Ballota nigra, Erigeron canadense, Arctium lappa;* K4: *Urtica dioica:* K3: *Cynodon dactylon, Lolium perenne, Poly-*

gonum lapathifolium, Chenopodium glaucum, Amaranthus retroflexus, Melandrium album, Conium maculatum, Solanum nigrum, Achillea millefolium incl. collinum, Artemisia vulgaris, Arctium tomentosum. — Wichtige Arten: *Cannabis sativa, Atriplex tatarica, A. hastata, Chenopodium hybridum, Ch. glaucum, Ch. urbicum, Amaranthus albus, Lepidium ruderale, Xanthium strumarium, Galinsoga parviflora, Arctium minus.*

Artenzahl: 99.

Konstitutionsspektrum: K5; 5, 0; K4: 1, 0; K3: 11, 0; K2: 25, 0; K1: 58%.

Bio-ökologisches Spektrum: Th: 53; H: 26; TH: 16; G: 3; Ph: 2%.

Verteilung der Florenelemente: Kont: 1, Pm: 1; M: 3; K: 17; Adv: 14; Cp: 7; Eua: 40; Eu: 11; Em: 6%.

Cytologische Daten: d: 53, 3; P: 38, 3; i: 8, 4%; d/P: 1, 39; (P)%: 46, 2%.

(Vgl. Braun-Blanquet u. De Leeuw 1936, Felföldy 1942, p. 119, Morariu 1943, p. 170.)

VI. Onopordion acanthii Braun-Blanquet 1926.

In diesen Verband gehören gleichfalls echte Ruderalien, doch zeigen diese bereits einen ausgeprägten Übergang zur Weidenvegetation (mechanisches Wirkungsplus!). Die typischste physiognomische Eigenheit dieser Verbände ist die absolute Herrschaft der Stechpflanzen. Sie sind xerophiler als die Arctionmitglieder.

13. *Xanthium spinosum-Ass.* Der ziemlich niedrig wachsende, sehr stachelige Verband dieses unangenehmen, eingeschleppten Unkrautes findet sich auf Weidewegen, wo der Boden durch den Verkehr aufgelockert ist. Auf Schweineweiden wird diese Auflockerung durch das Wühlen der Schweine besorgt.

Charakteristische Artenkombination: K5: *Malva neglecta, Xanthium spinosum;* K4: *Hyoscyamus niger, Solanum nigrum;* K3: *Hordeum murinum, Polygonum aviculare, Chenopodium vulvaria, Eryngium campestre, Xanthium strumarium.* — Wichtige Arten: *Cynodon dactylon, Diplotaxis muralis, Ononis spinosa, Euphorbia cyparissias, Heliotropium europaeum, Onopordum acanthium, Tragus racemosus, Tribulus orientalis* usw.

Artenzahl: 52.

Konstitutionsspektrum: K5: 3, 8; K4: 3, 8; K3: 9, 6; K2: 40, 4; K1: 42, 3%.

Bio-ökologisches Spektrum: Th: 53, 8; H: 30, 8; TH: 13, 5; G: 1, 9%.

Verteilung der Florenelemente: P: 1, 9; Pm: 3, 8; M: 7, 7; K: 21, 1; Adv: 5, 8; Cp: 7, 7; Eua: 32, 7; Eu: 11, 6; Em: 7, 7%.

Cytologische Daten: d: 53, 7; P: 37, 6; i: 8, 7%; d/P: 1, 43; (P)%: 36, 4%.

(Vgl. Felföldy 1942, p. 121, Morariu 1943, p. 183.)

14. *Carduus acanthoides-Ass.* Sie spielt eine ähnliche Rolle wie der Arctium lappa-Ass. und ist sehr verbreitet, schützt aber ihre Bewohner besser vor der Abweidung und dem Zertreten. Unter den Stechstauden verbleiben viel perennierende und hemitherophytische zweijährige Pflanzen. Es ist dies der einzige von mir studierte Arvidesertа-Verband, in dem der Th-Wert die 50% nicht erreicht (42, 1%). Die Carduus-Arten sind ausgesprochen zweijährig und weisen eher auf die Hemikryptophyten hin im Gegensatze zu dem eher überwinternden einjährigen *Onopordum* und dem immer therophytische *Xanthium*.

Charakteristische Artenkombination: K5: *Ballota nigra Carduus acanthoides:* K4: *Polygonum aviculare, Chenopodium album, Erigeron canadense, Achillea millefolium, Artemisia vulgaris;* K3: *Lolium perenne, Hordeum murinum, Urtica dioica, Atriplex tatarica, Arctium lappa, Onopordum acanthium, Lactuca serriola.*

Artenzahl: 81.

Konstitutionsspektrum: K5: 2, 5; K4: 6, 0; K3: 8, 4; K2: 9, 6, K1: 73, 5%.

Bio-ökologisches Spektrum: Th: 42, 1; H: 27, 7; TH: 25, 3; G. 3, 6; Ph: 1, 3%.

Verteilung der Florenelemente: Kont: 2, 5; Pm: 3, 6; M: 2, 5; K: 10, 8; Adv: 7, 2; Cp: 3, 6; Eu: 10, 8; Em: 8, 4%.

Cytologische Daten: d: 63, 7; P: 24, 5; i: 11, 8%; d/P: 2, 60; (P)%: 34, 3%.

(Vgl. Morariu 1939, 1943, p. 160, Felföldy 1942, p. 122.)

15. *Onopordum acanthium-Ass.* Die unter den ungünstigsten Umständen lebende, anspruchsloseste Stechruderalie. Sie kann abwechselnd mit dem vorhergehenden *Carduetum* vorkommen, und häufig scheint in der Tat lediglich der Zufall darüber zu entscheiden, welche Art zur Dominanz gelangt. Dennoch muss sie abgesondert besprochen werden, denn ihr ökologisches Spektrum unterscheidet sich vollends von dem des Carduetums. Der Th-Prozentwert fällt in die dem *Onopordion*-Verbande entsprechenden Werte von 50—60%.

Charakteristische Artenkombination: K5: *Polygonum aviculare,*

Malva neglecta, Ballota nigra, Erigeron canadense, Achillea millefolium, Onopordum acanthium; K4: *Cynodon dactylon, Lolium perenne, Hordeum murinum, Chenopodium album, Solanum nigrum, Xanthium strumarium, Artemisia vulgaris, Carduus acanthoides, Cichorium intybus;* K3: *Urtica dioica, Chenopodium vulvaria, Atriplex tatarica, Melandrium album, Reseda luteola, Marrubium vulgare, Verbascum phlomoides, Xanthium spinosum.*

Artenzahl: 70.

Konstitutionsspektrum: K5: 8, 4; K4: 12, 5; K3: 12, 5; K2: 11, 1; K1: 55, 5%.

Bio-ökologisches Spektrum: Th: 55, 5; H: 20, 8; TH: 22, 3; G: 1, 4%.

Verteilung der Florenelemente: P: 1, 4; Pm: 1, 4; M: 5, 5; K: 19, 4; Adv: 9, 7; Cp: 8, 4; Eua: 40, 3; Eu: 8, 4; Em: 5, 5%.

Cytologische Daten: d: 61, 8; P: 28, 1; i: 10, 1%; d/P: 2, 20; (P) %: 33, 9%.

(Vgl. Braun-Blanquet 1931, p. 29, Braun-Blanquet-Gajewski, Wraber, Walas 1936, p. 29, Libbert 1932, p. 39, Felföldy 1942, p. 124, Morariu 1943, p. 166).

VII. Polygonion avicularis Aichinger 1933.

Nach Morariu (1943, p. 150) zähle ich anstatt in das mediterrane Hordeion murini Br. — Bl. 1931 in das Polygonion die viel Therophyten enthaltenden Rasen mit den niedrigen Hecken der stark betretenen Wege und Wegränder.

16. *Poa annua-Ass.* Von den *Polygonion*-Mitgliedern lebt sie auf den verhältnismässig günstigsten Biotopen. Man findet sie auf feuchten, schattigen, dennoch betretenen, zuweilen kleinsteinigen Böden. Sie ist ein sehr typischer Rasen, der sich grosser Verbreitung erfreut.

Charakteristische Artenkombination: K5: *Poa annua, Polygonum aviculare, Plantago major;* K4: *Lolium perenne, Erigeron canadense;* K3: *Trifolium repens, Verbena officinalis, Taraxacum officinale.* — Wichtige Arten: *Hordeum murinum, Stellaria media, Capsella bursa pastoris, Medicago lupulina, Prunella vulgaris, Euphorbia peplus, Senecio vulgaris* usw.

Artenzahl: 52.

Konstitutionsspektrum: K5: 5, 8; K4: 3, 8; K3: 5, 8; K2: 15, 4; K1: 69, 2%.

Bio-ökologisches Spektrum: Th: 53, 8; H: 38, 5; TH: 7, 7%.

Verteilung der Florenelemente: Kont: 1, 9; K: 28, 9; Adv. 5, 8; Cp: 5, 8; Eua: 51, 9; Eu: 3, 8; Em: 1, 9%.

Cytologische Daten: d: 57, 5; P: 42, 5; i: — %; d/P: 1, 35; (P) %: 43, 1%;

(Vgl. Gams 1927, p. 334, Aichinger 1933, p. 61: *Lolium perenne — Poa annua*-Ass., Felföldy 1942, p. 126, 1943 a, p. 60.)

17. *Polygonum aviculare*-Ass. Eine in ganz Europa verbreitete (vgl. Gams 1927, p. 382) Gesellschaft entlang betretener Wege. Im Falle von Komplexen entlang von Wegen säumt immer dieser Verein den Rasen gegen die nackten, abgetretenen Flächen.

Charakteristische Artenkombination: K5: *Polygonum aviculare;* K4: *Lolium perenne, Hordeum murinum, Chenopodium album, Erigeron candense;* K3: *Plantago lanceolata, P. major, Taraxacum officinale. Poa annua, Trifolium repens, Convolvulus arvensis, Achillea collina.*

Artenzahl: 71.

Konstitutionsspektrum: K5: 1, 4; K4: 5, 5; K3: 4, 2; K2: 9, 7; K1: 79, 2%.

Bio-ökologisches Spektrum: Th: 57; H: 30, 5; TH: 6, 9; G: 4, 2; Ch: 1, 4%.

Verteilung der Florenelemente: P: 1, 4; Pm: 1, 4; M: 8, 4; K: 15, 2; Adv: 15, 2; Cp: 9, 7; Eua: 41, 6; Eu: 8, 4; Em: 1, 4%.

Cytologische Daten: d: 62, 2; P: 29, 1; i: 8, 7%; d/P: 2, 14; (P) %: 39, 7%. (Vgl. Gams 1927, p. 382, Soó 1940, p. 42, Hargitai 1940, p. 27, Felföldy 1942, p. 127, 1943 a, p. 59, Morariu 1943, p. 157.)

18. *Medicago lupulina-Ass.* Die Rolle der kleinen Leguminosen in der Vegetation der betretenen Ruderalien wurde bereits von Drude (1896, p. 422) betont. Ein solcher typischer Verein ist die *Medicago lupulina*-Ass., in der neben absolut dominierendem *M. lupulina* die Trifolien *(fragiferum, repens, campestre, pratense, arvense)* charakteristisch sind. Ökologisch kann ich über sie folgendes aussagen: Etwas feuchter, in jedem Falle aber kleinsteiniger Boden, mässige Betretung und die einmalige intensive Störung des Biotops zu Sommeranfang, (z. B. die zu Sommeranfang gereinigten Wege von Parks, Promenaden, Wasserrisse, erste Berasung von mit Wasser bespültem kiesigem Schutt, bisweilen auf dem Kies von Eisenbahndämmen).

Charakteristische Artenkombination: K5: *Medicago lupulina, Trifolium repens, T. pratense, Plantago lanceolata, Erigeron canadense, Taraxacum officinale;* K4: *Poa annua, Polygonum aviculare, Convolvulus arvensis;* K3: *Setaria glauca, Poa pratensis incl. angustifolia, Bromus*

mollis, Lolium perenne, Agropyron repens, Trifolium fragiferum, Lotus corniculatus, Anagallis arvensis, Verbena officinalis, Plantago major. Wichtige Arten: *Diplotaxis muralis, Capsella bursa pastoris, Trifolium campestre, T. arvense, Lathyrus tuberosus, Melilotus officinalis, Ononis spinosa, Achillea collina.*

Artenzahl: 85.

Konstitutionsspektrum: K5: 7, 0; K4: 3, 4; K3: 11, 6; K2: 14, 0; K1: 64, 0%.

Bio-ökologisches Spektrum: Th: 57, 7; H: 29, 4; TH: 8, 2; G: 4, 7%.

Verteilung der Florenelemente: Kont: 3, 5; P: 1, 2; Pm: 2, 4; M: 9, 5; K: 16, 4; Adv: 3, 5; Cp: 5, 9; Eua: 45, 8; Eu: 8, 2; Em: 2, 4; end: 1, 2%.

Cytologische Daten: d: 54, 1; P: 35, 3; i: 10, 6%; d/P: 1, 53; (P)%: 39, 5%.

(Vgl. Drude 1896, p. 422, Felföldy 1942, p. 129, 1943 a, p. 61.) 129, 1943 a, p. 61.)

19. *Hordeum murinum-Ass.* Nebst Polygonetum unsere typischste stark betretene Gesellschaft. Sie lebt indessen auf weitaus ungünstigeren Plätzen als die vorhergehende und ist fast immer eine Anfangs-, eine Pioniervergesellschaftung. Sie lebt am häufigsten auf solchen kotigen Stellen, wo die Vegetation im Vorfrühling von Tieren zerstört wurde, und im Mai entwickelt sich dieser primitive, ephemere Rasen. Hordeum murinum ist eine überwinternde einjährige Pflanze, die ab dem Vorfrühling bis etwa Mai in sehr grossen Mengen in Vereinen entlang von Wegen stellenweise auffällt. Eine spätere Untersuchung nach dem Verblühen des Hordeums belehrt indessen, dass wir es eigentlich nur mit dem Vorfrühlings- und Sommeranfangs-Aspekt (*Hordeum murinum*-Asp.) von *Lolium*- oder *Cynodon*-Rasen zu tun hatten.

Charakteristische Artenkombination: K5: *Lolium perenne, Hordeum murinum, Polygonum aviculare;* K4: —; K3: *Apera spica-venti, Chenopodium album, Atriplex oblongifolia, Portulaca oleracea, Stellaria media, Ballota nigra, Erigeron canadense.* Wichtige Arten: *Atriplex tatarica, Trifolium repens, Convolvulus arvensis, Taraxacum officinale.*

Artenzahl: 41.

Konstitutionsspektrum: K5: 7, 3; K4: —; K3: 17, 1; K2: 14, 6; K1: 61, 0%.

Bio-ökologisches Spektrum: Th: 63, 4; H: 22, 0; TH: 12, 2; G: 2, 4%.

Verteilung der Florenelemente: Kont: 2, 4; Pm: 2, 4; M: 2, 4; K: 24, 4; Adv: 9, 8; Cp: 9, 8; Eua: 43, 9; Em: 4, 9%.

Cytologische Daten: d: 56, 6; P: 35, 4; i: 8, 0%; d/P: 1, 60; (P)%: 40, 5%.

(Vgl. Libbert 1932, p. 39, Braun-Blanqet 1931 (Aus dem Mediterran!), Tüxen 1937, p. 22, Soó 1940, p. 42, Felföldy 1942, p. 131, Morariu 1934, p. 150.)

VIII. Secalinion medioeuropaeum Tüxen 1937.

Hierhin gehören die typischsten anthropogenen Pflanzenvereine, die von der landwirtschaftlichen Arbeit selbst produziert werden. Der Forscher stösst hier auf viel ungelöste Probleme, von denen die wichtigsten sind: das Verhältnis von Ruderal- und Kulturpflanzen, ihre gegenseitige Wirkung aufeinander, der soziologische Zusammenhang zwischen Saaten und Stoppelfeldern; (die Stoppelvegetation ist eigentlich nur eine chronologische Variation, ein Aspekt der Saat) usw. usw.

20. *Setaria glauca-Stachys annua-Ass.* Sie ist der typische Stoppelverein unseres kontinentalen Klimas. Diese Feststellung ist in dem Sinne zu modifizieren, dass die Setarieta für die Örter typisch sind, deren Rhytmus dem der Stoppel ähnlich ist, d. h. intensive Störung im Anfange der Vegetationsperiode, sodann mehr oder weniger ruhiger Entwicklungsgang. Die untenstehend besprochene Gesellschaft ist die spezielle Assoziation der Stoppelfelder.

Charakteristische Artenkombination: K5: *Setaria glauca, Fagopyrum convolvulus, Chenopodium album, Consolida regalis, Diplotaxis muralis, Medicago lupulina, Euphorbia falcata, Anagallis arvensis, Convolvulus arvensis, Ajuga chamaepithys, Stachys annua, Kickxia elatine, Erigeron canadense, Sonchus arvensis;* K4: *Polygonum aviculare, Nigella arvensis, Cerinthe minor, Sideritis montana, Galeopsis angustifolia, Melampyrum barbatum, Cirsium arvense:* K3: —. — *Wichtige* Arten: *Vicia pannonica, Euphorbia exigua, Thymelaea passerina, Papaver rhoeas, Kickxia spuria, Centaurea cyanus, Crepis rhoeadifolia.*

Artenzahl: 83.

Konstitutionsspektrum: K5: 16, 6; K4: 8, 3; K3: —; K2: 10, 8; K1: 64, 3%.

Bio-ökologisches Spektrum: Th: 64, 4; H: 23, 8; TH: 5, 9; G: 5, 9%.

Verteilung der Florenelemente: Kont: 3, 5; P: 1, 2; Pm: 5, 9;

M: 15, 4; end: 1, 2; K: 11, 9; Adv: 7, 1; Cp: 5, 9; Eua: 34, 5; Eu: 13, 0%.

Cytologische Daten: d: 54, 5; P: 30, 8; i: 14, 7%; d/P: 1, 77; (P)%: 26, 6%.

(Vgl. Soó 1932, p. 119, Bojkó 1934, p. 710. Ujvárosi 1937, p. 200, Felföldy 1942, p. 130.)

21. *Setaria glauca-Digitaria sanguinalis-Ass.* Sie steht den zur Mobilideserta gehörenden *Bromion tectorum*-Verbänden (Soó 1940, p. 35) nahe, ist aber ausgesprochen ruderal. Sie erscheint hauptsächlich auf Sand nach künstlichem Aufbruch mit ihren vielen Unkrautbegleitern und ohne der für *Bromion* fast immer charakteristischen Moosschicht *(Syntrichia, Ceratodon, Barbulae* usw.). Sie kann auch auf gebundenem Boden leben, wie ich dies in Tihany studieren konnte. Sie ist eine echte ephemere, einsommerige Gesellschaft nicht allein, weil ihre Mitlgieder Therophyten sind, sondern auch aus dem Grunde, dass sie ohne neuere Störung in jedem Falle im Anfange der zweiten Vegetationsperiode verschwindet um einem dynamischeren Verein *(Lolion!)* den Platz zu räumen. Sie erscheint auf den hauptsächlich im Frühling gereinigten, gejäteten Wegen von Promenaden, Parks, wenn dessen Trockenheit die Entwicklung des *Poa-annua*-Rasens behindert.

Charakteristische Artenkombination: K5: *Digitaria sanguinalis, Setaria glauca, Rumex acetosella, Polygonum aviculare, Chenopodium album, Erigeron canadense;* K4: *Melandrium album, Trifolium arvense, Crepis setosa;* K3: *Setaria viridis, Apera spica venti, Eragrostis minor, Cannabis sativa, Crepis rhoeadifolia.* — Wichtige Arten: *Eragrostis pilosa, Bromus mollis, B. tectorum, Portulaca oleracea, Arenaria serpyllifolia, Oenothera biennis, Scabiosa ochroleuca.*

Artenzahl: 37.

Konstitutionsspektrum: K5: 15, 8; K4: 7, 8; K3: 15, 8; K2: 28, 9; K1: 31, 6%.

Bio-ökologisches Spektrum: Th: 65, 9; H: 10, 5; TH: 10, 5; G: 7, 8; Ch: 5, 2%.

Verteilung der Florenelemente: Kont: 2, 6; P: 2, 6; M: 2, 6; K: 31, 7; Adv: 13, 2; Cp: 7, 8; Eua: 31, 7; Eu: 2, 6; Em: 5, 2%.

Cytologische Daten: d: 48, 1; P: 32, 2; i: 19, 7%; d/P: 1, 49; (P)%: 50, 0%.

(Vgl. Felföldy 1942, p. 134, 1943a. p. 57).

22. *Portulaca oleracea-Ass.* Sie ist die auf extremstem, speziellstem Standorte lebende, verarmteste, sich aber mit bedeutender Gesetzmäs-

sigkeit wiederholende Gesellschaft. Sie ist in Weingärten, Küchengärten, hauptsächlich auf trockenem sandigem Boden zu finden. Ausser durch *Portulaca* wird sie von einsommerigen Gräsern und solchen Ausdauernden gekennzeichnet, deren in die Erde versteckte überwinterte Organe nach dem Hacken zu spriessen vermögen *(Cynodon, Convolvulus arvensis* usw.).

Charakteristische Artenkombination: K5: *Digitaria sanguinalis, Eragrostis minor, Portulaca oleracea, Convolvulus arvensis;* K4: *Stellaria media, Senecio vulgaris;* K3: *Setaria glauca, Amaranthus retroflexus, Solanum nigrum, Erigeron canadense.* — Wichtige Arten: *Erodium cicutarium, Lamium amplexicaule, Cynodon dactylon* usw.

Artenzahl: 23.

Konstitutionsspektrum: K5: 17, 4; K4: 8, 7; K3: 17, 4; K2: 13, 0; K1: 43, 5%.

Bio-ökologisches Spektrum: Th: 91, 2; H: 4, 4; G: 4, 4%.

Verteilung der Florenelemente: K: 56, 5; Adv: 13, 0; Cp: 4, 4; Eua: 21, 7; Eu: 4, 4%.

Cytologische Daten: d: 29, 4; P: 48, 5; i: 22, 1%; dP: 0, 60; (P)%: 52, 7%.

(Vgl. Felföldy 1942, p. 135).

IX. Polygono-Chenopodion Koch 1926.

23. *Setaria glauca-Echinocloa crus galli-Ass.* Eingentlich gehören in unserem Vaterlande in diesen Verein alle an feuchteren Stellen lebenden Ruderalassoziationen, die man unter dem Namen Olitorion zu erwähnen pflegt. Die typischste Entwicklung dieser Gesellschaft ist in Hackkulturen gegen den Herbst zu zu finden. Bei uns herrschen in den Hackkulturen zufolge des kontitentalen Makroklimas trockenere Verhältnisse als im Westen, deshalb kann ich den Namen *Panico-(Echinochloeto)-Chenopodietum,* weil nicht charakteristich, nicht gebrauchen (vgl. Braun-Blanquert 1921, Libbert 1932, p. 33, Tüxen 1937, p. 29, Soó 1940, p. 42). Zufolge der Trockenheit sind nähmlich die Chenopodien nicht konkurrenzfähig gegen *Setaria glauca (Chenopodium album* A—D 1—2 (3), *Setaria glauca* (2)—3; eine andere *Chenopodium*-Art kommt in meinen Aufnahmen denn auch nicht vor!)

Charakteristische Artenkombination: K5: *Echinochloa crusgalli, Setaria glauca, Chenopodium album, Convolvulus arvensis, Erigeron canadense;* K4: *Polygonum lapathifolium, Amaranthus retroflexus, Sonchus arvensis;* K3: *Equisetum arvense, Reseda lutea, Hibiscus*

trionum, Mentha longifolia, Plantago major, Xanthium strumarium, Cirsium arvense, Sonchus asper.

Artenzahl: 72.

Konstitutionsspektrum: K5: 6, 9; K4: 4, 1; K3: 11, 1; K2; 16, 6; K1: 61, 2%.

Bio-ökologisches Spektrum: Th: 69, 5; H: 19, 4; TH: 5, 5; G: 5, 5%.

Verteilung der Florenelemente: Kont: 1, 4; Pm: 1, 4; M: 1, 4; K: 25, 0; Adv: 8, 4; Cp: 4, 2; Eua: 36, 0; Eu: 13, 8; Em: 1, 4%.

Cytologische Daten: d: 45, 8; P: 41, 5; i: 12, 7%; d/P: 1, 95; (P)%: 34, 4%.

III. Cytogeographischer Teil.

Die Ergebnisse der Polyploidenforschung können vom pflanzengeographischen Gesichtspunkte aus wie folgt zusammengefast werden:

1. Polyploide Pflanzen vermögen in der Natur unter extremen Lebensumständen zustande zu kommen (Kälte-Wärmewirkung, ungünstige Hydratur usw.).

2. In extremen Klima ist der Prozentwert der Polyploiden höher als in der gemässigteren Zone.

3. Innerhalb des Rahmens irgendeiner Gattung oder Art ist die geographische Verbreitung der polyploiden Formen grösser als die der Diploiden. Demzufolge zieht die Entstehung der Polyploiden eine Verbreitung der Art in weiterem Kreise gegen ungüstigere Gebiete zu nach sich.

4. Obige Gesetzmässigkeiten sind nicht allein im Falle makroklimatischer Unterschiede studierbar, vielmehr auch in der Zusammensetzung der Vegetation von Biotopen mit verschiedenartiger ökologischer Einstellung.

Gestützt auf die Ergebnisse von Hagerup 1932, Rohweder 1936, Tischler 1937, Wulff 1937, Tarnawschi 1938, Löve-Löve 1942 u. A. versuchte ich die cytologische Analyse der pannonischen Ruderalassoziationen. Ich erachte für richtig die Meinung Arwidssons (1938), dass die Schrifttumsangaben nicht geeignet sind für die genaue Analyse, deshalb habe ich diese erst nach Bestimmung einer sehr grossen Chromosomenzahl durchgeführt; dennoch musste ich viele Angaben aus dem Schrifttum übernehmen.

Methodik s. S. 128.

Die Wichtigkeit der Polyploiden im Aufbau der Ruderalvegetation wurde bereits von Tischler (1937) betont. Er fand in der eingebürgerten Halligenflora eine leichte Steigerung des Gehaltes an Polyploiden gegenüber dem benachbarten Lande. Ganz deutlich wird der ökologische Wert der Polyploiden bei den Unkräutern; diejenigen, die sich gegenüber der Umwelt durchzusetzen vermochten und jetzt völlig eingebürgert sind, weisen einen sehr hohen Gehalt an Polyploiden auf im Gegensatz zu den „nich wirklich eingebürgerten".

Eine genaue cytogeographische Analyse eines Pflanzenvereines liesse sich nur durchführen, wenn mehrere Individuen aller Arten cytologisch untersucht würden. Dazu gehörte indessen z. B. im Falle eines Landes eine ganze Institution, deshalb begnügen wir uns mit der Untersuchung 1. der Arten unbekannter Anzahl, 2. deren, die laut dem Schrifttum mehrere Chromosomenrassen enthalten oder 3. der vielerlei Zahlen enthaltenden Arten. Auch so bleiben noch viele Pflanzen unbekannt (33, 9% der pannonischen Flora, etwa 700 Arten!). Der statistische Fehler, den diese unbekannten Pflanzen verursachen, lässt sich auf die Weise ausschalten, dass man das Verhältnis der Diploiden und Polyploiden in den verschiedenden Pflanzengesellschaften miteinander vergleicht. Dies kann auf zweierlei Weise bewerkstelligt werden: 1. indem man von der Zahl sämtlicher Arten die unbekannten abzieht und aus dem Rest den Prozentwert der Diploiden und Polyploiden: (d)% und (P)% berechnet; (die Klammern bedeuten, dass sie auf den Rest ohne Unbekannte bezogene Werte sind). Die meisten Verfasser arbeiten auf diese Weise. 2. Für genauer halte ich den aus der Gesamtzahl berechneten Quotienten d/P, der der Polyploidenzahl umgekehrt proportional ist.

Zur Ausschaltung der statistischen Streuung kann die von Tischler empfohlene Formel benutzt werden, nach der der mittlere Fehler $M_1 = a \pm \frac{a.\ (100 - a)}{n}$ ist; darin bedeutet „a" den Prozentwert der polyploiden Arten, „n" den absoluten Wert. Mit je mehr bekannten Arten man arbeitet, umso geringer wird der ±-Wert. Im Falle von Pflanzenassoziationen halte ich es aber für besser aus dem d- und P%-Werte mehrerer Aufnahmen den Mittelwert zu errechnen (M%) und aus diesem den Quotienten d/P festzustellen. Meine Daten und die d/P-Linie von Abb 1. haben diesen Ursprung; in die Abbildung habe ich zweks besserer Veranschaulichung das 10fache der Verhältniszahl (10 d/P) eingezeichnet.

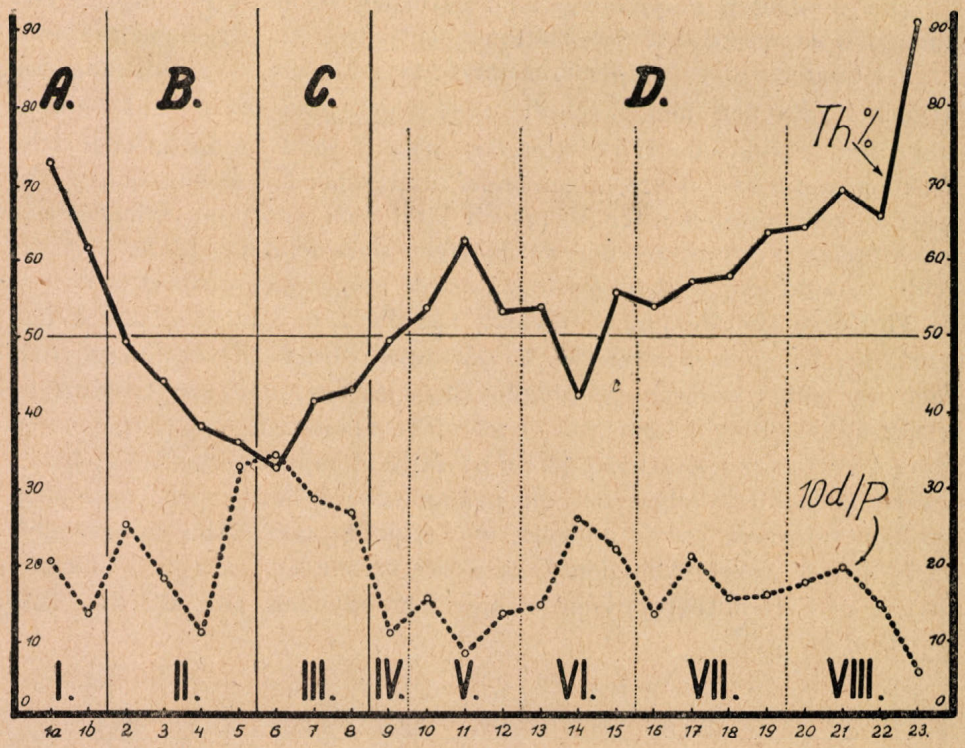

Abb. 1. Erklärungen im Texte.

Betrachten wir nunmehr Abb. 1. Die Linie d/P bezeugt, dass die relativ die meisten Polyploiden enthaltenden Vereine die *Portucala oleracea* und die *Solanum nigrum*-Ass. sind. Beide Standorte werden von intensiver und dauernder Störung gekennzeichnet, das Leben der Vegetation ist ein ganz kurzes, die Sukzession beginnt immer wieder von vorn. (Gerade diese dauernde Störung stabiliert die Stadien, indem sie dieselben auf den Rang von Assoziationen erhebt. Vg. Felföldy 1942, p. 94.) Es ist der gleiche Fall wie der von Tischler's Halligflora. Sodann folgen die Vereine von *Potentilla anserina, Bidens tripartita, Juncus bufonius-Potentilla* und *Poa annua*. Eine auffallende Unregelmässigkeit verraten diese auch im Falle des Vergleichs mit der Th-Linie! Nach unseren bisherigen Kenntnissen weichen sie etwas von der Norm ab. Es sind feuchtigkeitliebende, stark nitrophile Gesellschaften, deren hoher Polyploid-Wert auch mit der Stickstoffwirtschaft zusammenhängen kann. Ein der weiteren Forschung harrendes Problem!

Die wenigsten Polyploiden sind natürlich in den strauchigen, dornigen und immergrünen Assoziationen enthalten als Ergebnis der milderen Standorte der *Sempervirentiherbosa* und der Schutzwirkung der Sträucher, Stauden und Stechpflanzen; (hier sind nicht mikroklimatische und edaphische Wirkungen am Werke, sondern die mechanische Wirkung weidender Tiere, kommender und gehender Menschen).

Ein sehr interessanter Vergleich lässt sich anstellen zwischen der prozentualen Verbreitung der Einjährigen und dem d/P Linie. Wie ersichtlich, sind die beiden Werte einander umgekehrt proportional: wo die Th-Kurve ihr Maximum erreicht, hat d/P sein Minimum und umgekehrt. Es bieten sich zweierlei Erklärungen dieser Erscheinung dar. Nach der einen besteht die Möglickeit, dass es unter den einjährigen Pflanzen viel Polyploiden gibt, was nicht allein mit den Ergebnissen älterer Verfasser (z. B. Hagerup) in Widerspruch stände, vielmehr erweist auch die Analyse der die pannonische Pflanzendecke aufbauenden Lebensformen etwas anderes, was Tab. I und Abb. 2 bezeugen. Nach diesen weisen von den Lebensformverbänden nur die Chamaephyten und Hemitherophyten weniger Polyploiden auf als die Einjährigen.

	G	HH	Ch	TH	Th	H	Ph
d	36,0	38,7	26,0	52,5	46,0	37,2	46,4
P	26,6	33,4	12,0	21,8	23,2	27,6	25,8
i	37,4	27,9	62,0	25,7	30,8	35,2	27,8
(P) %	42,5	46,3	31,6	29,4	33,5	42,6	35,9
d/P	1,36	1,16	2,16	2,38	1,98	1,34	1,79

Tab. I. Cytologische Verhältnisse der Lebensformen in der ungarischen Vegetation (Abkürzungen s. S. 129.)

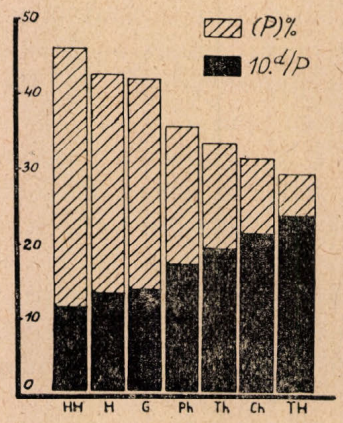

Abb. 2. Die Polyploidverhältnisse der pannonischen Lebensformen, zugleich Vorführung der umgekehrten Proportion von (P)% und d/P. (Abkürzungen s. S. 129.)

Somit verbleibt die andere Erklärung: Den extremen Lebensumständen hat sich die therophytische Lebensform am besten angepasst, weil diese im widerstandsfähigsten Zustande als Samen die ungünstigste Zeit überdauert hat. Findet man, dass die relativ meisten Polyploiden in den die meisten Therophyten enthaltenden (also unter den extremsten Verhältnissen lebenden) Assoziationen vorkommen, so kann ruhig behauptet werden, dass die Selektion dieser extremen Verhältnisse die

Gebiet, Biotop od. er Pflanzengesellschaft	(P) %	Verfasser
Dänemark	51,9	Löve-Löve 1942
Finnland	54,4	
Norwegen	53,9	
Schweden	53,6	
Island	54,5	
Spitzbergen	c. 80	Flovik 1940
Kaukasus (Alp.)	50,0	Sokolowskaja u. Strelkowa 1938, 1940 ap. Löve-Löve 1942
Altai (Alp.)	65,0	
Pamir (Alp.)	85,0	
Schleswig-Holstein	46,0	Tischler ap. Löve-Löve 1942
Ungarn (Pannonicum)	39,4	Felföldy 1946 (unveröffentl.)
Schleswig-Holstein (Haloph.)	55,6	Wulff 1937
Rumänien (Haloph.)	26,3	Tarnawschi 1938
Hortobágy: Trockenweiden auf Szikböden (Festucet. pseudovinae)	46,5	Felföldy 1946 (unveröff.)
Hortobágy: Blind-Szik (Camphorosmetum)	59,8	
Dünevegetation:		Rohweder 1937
An windausgesetzten Spitzen	90,0	
Auf primären und sekundären Dünen	75,0	
In windgeschützten Tälern	65,0	
Portulaca oleracea-Ass.	52,7	Felföldy 1946
Solanum nigrum-Ass.	50,0	
Setaria-Digitaria-Ass.	50,0	
Cynodon dactylon-Ass.	27,9	
Sambucus ebulus-Ass.	29,8	
Polygonum aviculare-Ass.	39,7	
Arctium lappa-Ass.	46,2	
Bidens tripartita-Ass.	45,0	
Lolium perenne-Ass.	31.1	

Tab. II. Prozentwert der Polyploiden in verschiedenen Gebieten, Biotopen und Pflanzengesellschaften.

Polyploiden in den fraglichen Verein drängt. Der Parallelismus der extremen Lebensumstände und des Polyploid-Prozentwertes ist demnach nicht allein im Falle erheblicher zonaler Unterschiede oder besonders extremer Standorte (salzige, kalkhaltige und sandige Böden) studierbar, sondern auch in der Zusammensetzung der einzelnen Pflanzenvereine. Die Relation d/P oder aber der Prozentwert der Polyploiden sind also Daten, die über die allgemeine ökologische Einstellung des fraglichen Pflanzenvereins Aufschluss erteilen.

Zum Beschluss teile ich in Tab. II einige lehrhafte Beispiele auf Grund des Schrifttums und meiner eigenen Daten mit.

IV. METHODIK, ABKÜRZUNGEN

Die Aufnahmen des soziologischen Teiles erfolgten mit einem 2x2 m-Quadrat nach den Methoden von Braun-Blanquet (1918). Die Charakterarten spielen in der mosaikartig abwechslungsreichen Vegetation unseres Vaterlandes keine so wichtige Rolle; erheblich ausdrucksvoller ist die charakteristische Artenkombination, die die ständigen (K5—3), durch ihre Masse kennzeichnenden oder von anderen Gesichtspunkten aus (Charaktere des Standortes, seltene interessante Arten oder auffalend treue) wichtigen Arten enthält („wichtige Arten"). Das Problem der Verbands-, Assoziations- und Standortcharakterarten wird van Weiteren Forschungen erklärt werden (vgl. Felföldy 1943 b).

Die Spektren habe ich auf Grund von 20 Aufnahmen berechnet.

Bei der Feststellung der Lebensformen habe ich die Arbeit Raunkiaer's (1934) mit entsprechender Kritik benutzt. In unserem kontinentalen Klima erfrieren viel Ch-Elemente und überwintern als Hemikryptophyten (z. B. *Ballota nigra, Solanum dulcamara* usw). Die Zweijährigen reihe ich im Gegensatz zu Raunkiaer u. A. nicht unter die Hemikryptophyten, behandle sie vielmehr abgesondert unter dem Namen Hemitherophyton, weil sie bei uns ausgesprochen auf die Einjährigen hinweisen. Auf trockenerem Biotop überwintern viel HH-Elemente als Geophyten usw.

Die Einteilung der Florenelemente beruht auf den Arbeiten von Máthé (1940, 1941).

Die cytologischen Untersuchungen habe ich z. T. in mit Carnoy Lösung fixierten Knospen nach dem Karminessigsäuren-Schnellverfahren, z. a. T. in mit Nawaschin-Lösung fixierten Wurzelspitzen (in Paraffin eingebettete 12 μ dicke Schnitte!) mittels Clausen- Oehlkers-

Johannsenscher Gentianaviolettfärbung durchgeführt. Diese meinen Angaben werde ich später veröffentlichen.

Die cytologischen Daten wurden aus dem Mittelwerte der Angaben von 20 Aufnahmen berechnet.

Die Erklärung der Abkürzungen ist die folgende:

A—D = Abundanz-Dominanz (Vgl. Braun-Blanquet 1926).
Adv = adventive Florenelemente
Ch = Chamaephyten
Cp = circumpolare Elemente
d = diploid
(d)% = der Prozentwert wird aus der Summe P+d berechnet
end = endemische Elemente
Em = mitteleuropäische Elemente
Eu = europäische Elemente
Eua = eurasiatische Elemente
G = Geophyten
H = Hemikryptophyten
HH = Hydro-Helophyten
i (ignotus) = Chromosomenzahl unbekannt
K = Kosmopoliten
K1—5 = Konstanzwert (Vgl. Braun-Blanquet l. c.)
K = kontinentale Elemente
M = mediterrane Elemente
P = pontische Elemente
P = (im cytologischen Spektrum) polyploid
(P)% = der Prozentwert wird aus der Summe P+d berechnet
Ph = Phanerophyten
Pm = pontisch-mediterrane Elemente
Th = Therophyten
TH = Hemitherophyten (zweijährige Pflanzen).

Der Verfasser dankt Herrn dr. Moritz Weiss für die deutsche Übersätzung und Herrn Prof. dr. R. von Soó für die zahlreiche wertvolle Ratschläge.

V. SCHRIFTTUM.

Aichinger E. 1933: Pflanzensoziologie 2. (Jena) — Allorge P. 1922: Les associations végétals du Vexin Francaise (Nemour) — Arwidsson Th. 1938: Sv. Bot. Tidskr. 32. p. 191. —

Bartsch J. u. M. 1940: Pflanzensoziologie 4. (Jena) — Beger H. 1930: Abderhalden: Handb. d. Biol. Arbeitsmeth. IX. 5. p. 511. — Bojko H. 1933. Beih.

z. Bot. Cbl. 50. 2. p. 207. — 1934: l. c. 51. p: 600 — Braun-Blanquet J: 1928: Pflanzensoziologie (Zürich) — 1931: Com. SIGMA 25. — Braun-Blanguet, Gajewski W., Wraber M., Walas J. 1936: Prodr. d. Pflanzengesellchaften 3. (Montpellier) — Braun-Blanquet J. u. De Leeuw W. C. 1936: Com. SIGMA 50. p. 1. —

Drude O. 1896: Deutschlands Pflanzengeographie (Berlin) —

Felföldy L. 1942: Acta Geob. Hung. 5. p. 87. — 1943 a: Magy. Biol. Kut. Munk. 15. p. 42. — 1943 b: Növényszociológia (Debrecen). Flovik K. 1940: Hered. (Lund) 26. p. 32. —

Gams H. 1927: Beitr. z. Geobot. Landesauin. d. Schweitz 15. — Geitler L. 1940: Schnellmethoden der Kern u. Chromosomenuntersuchung (Berlin). — Hagerup O. 1932: Hered. (Lund) 16. p. 19. — Hargitai Z. 1940a: Bot. Közl. 37. p. 212. — 1940 b: Acta Geob. Hung. 3. p. 27. —

Klika J. 1935: Veda Prirodni 16. p. 119. — Koch W. 1926: Jahrb. St. Gail. Nat. Ges. 61. p. 28. —

Libbert W. 1930: Mitt. Florist.-Soziol. Arbeitsgem, in NiederSachsen 2. p. 104. — 1932: I. Verh. Bot. Ver. Brandenburg 74. p. 10. — Löve A. u. Löve D. 1942: Botaniska Not. 1942. p. 19. —

Magyar P. 1933: Erd. Lapok. 72. p. 281. — Máthé I. 1940: Acta Geob. Hung. 3. p. 110. — 1941: l. c. 4. p. 85. — Moor M. 1936: Beitr. z. Geobot. Landesaufn. d. Schweitz 20. p. 1. — Morariu I. 1939: Archiva Somesana 25. — 1943: Bull. Grad. Muz. Bot. Univ. Cluj (Timisoara) 23. 3—4. p. 131. —

Oehlkers F. 1940: Z. Bot. 30. p. 24. —

Pénzes A. 1942: Budapest élővilága (Budapest) — Pfeiffer H. 1937: Beih. z. Bot. Cbl. 57. p. 599. — 1940: Beih. z. Bot. Cbl. 60. 2. p. 124. — Polgár S, 1912: Magy. Bot. Lapok 11. p. 308. —

Raunkiaer C. 1934: Danks Ekskursions-Flora (Kobenhavn). — Rohweder H. 1936: Beih. z. Bot. Cbl. 54. p. 507. — 1937: Planta (Berl.) 27. p. 500. —

Schwickerath M. 1940: Ber. D. Bot. Ges. 58. p. 5. — Soó R. 1931—32: Magy. Biol. Kut. Munk. 4. p. 293. u. 5. p. 118. — 1933: Debr. Szemle 1934. p. 26. — 1936: Fedde Rep. 39. p. 352. — 1940: Nova Acta Leop. 9. 56. p. 42. — 1945: Növényföldrajz (Budapest). —

Tarnawschi I. T. 1938: Bull. fac. Sci. Cernauti 12. p. 68. — Tischler 1927—1939: Tab. Biol. IV—XVI. — 1937: Cytologia (Tokyo) Fujii Festschr. p. 162. — Tüxen R. 1937: Die Pflanzengesellschaften Nordwestdeutschlands (Hannover). —

Ujvárosi M. 1937: Tisia (Acta Geob. Hung.) 2. p. 194. — 1940: Tisia (Acta Geob. Hung.) 4. p. 32. —

Wulff H. D. 1937: Jb. wiss. Bot. 84. p. 812. —

Zólyomi B. 1937: Bot. Közl. 34. p. 169. —

Tihany, 1946 nov. 1.

FASCIATIONS AND KINDRED TERATISMS INDUCED IN PLANTS BY POLYPLOIDOGENIC AGENTS.

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With 1 Table and 2 Figures in the Text and 5 Photographs.

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INTRODUCTION.

Long before Aldrovandi's "Monstrorum Historia" (1642), ever since antiquity, and up to our day, many instances of teratism have been reported in plants and animals. Amongst these anomalies fasciation and other forms of fusion (coalescence) are of fairly frequent occurrence in nature, and especially so in the vegetable kingdom (Schlotterbeck 1755, Moquin—Fandon 1915—16, Küster 1926, Georgescu 1926, Strohl 1929.) etc.). Few examples are known, however, of the *artificial* and especially of the chemical induction of such aberrant forms.

Pathologic conditions being a frequent source of these, the discovery of the oncogenic action of colchicine in plants, made by Havas (1936), and of the nuclear aberrations caused by the drug, as first shown by the Brussels school (Dustin—Havas 1937), threw the field open to the application of new methods and disclosed increased possibilities for the experimental induction of teratisms both by means of colchicine and other polyploidogenic substances (Rybin 1939, Krug 1942, Levan 1945.).

The evolutionary significance of both oncogeny and polyploidy being well known (De Quervain 1913, Lieske 1928, Kostoff 1933, Müntzing 1936, Dobzhansky 1939; Havas 1942a., 1942b.). the anomalies obtained by the use of polyploidizing agents seem doubly worth recording. On the other hand, although the fasciations and kindred phenomena to be described were mostly connected with tumour

formation and chromosome duplications, it will be seen that these are by no means a "conditio sine qua non" of the production of such structures.

EXPERIMENTAL.

1. WHEAT.

a.) Fasciation of roots (colchicine). Pure line Wilhelmina wheat and a Hungarian variety of unknown origin were germinated in Petri dishes on filter paper moistened with 2:1000, 1:1000, 1:2000 and 1:5000 solutions of colchicine. The treatments lasted from 12 to 14 days. The Figures show two types of fasciations obtained: Fig 1. pictures a root divided at the distal end and fusioned at the proximal end, while Fig. 2. shows the inverse processus. Such roots were observed in 9 cases out of about 4500, most of them in solutions of 1:2000. In 7 out of these 9 cases the coalescence was produced in roots showing the typical colchicine tumefaction, and all of these were tetraploid. In two cases, however, the fusion of colchicine treated normal filiform roots was also observed. Fasciation of the root hairs was found very often in both tumefied and normal-looking colchicine-treated roots. (HAVAS 1940.).

Various polyploidizing substances. Two fasciated and tumefied roots were found in about 400 plants treated with *phenyl-urethane* (1:5000), and fasciated root hairs were very frequent. Similar conditions were found in seedlings of wheat and corn treated with *acenaphtene, ethyl-mercury-phosphate* (HAVAS 1945) and *benzpyrene* (HAVAS and MENDELEEFF 1937.).

Other agents. It may be further mentioned that coalescence of roots and root hair was also encountered in wheat seedlings treated with such heterogenous substances as *Viscum album* (HAVAS, 1937. *Beta vulgaris* and *Derris* preparations and a *liposarcoma extract* (HAVAS—MENDELEEFF 1937.) and, although polyploidy was not observed as a consequence of these treatments none of the treated roots was normal, while coiled and ageotropic morphisms were frequent, resembling "pre-cancerous states" (HAVAS 1942.).

b.) Fasciation of shoots (colchicine). This anomaly was less frequent, 6 having been encountered in about 4500 treated seedlings. The coalescent part of these seedlings was two to three times thicker than that of the controls, measured at the same level, and their height did not exceed one-third of these last. In spite of the fact that two of the

fasciated plumules arose from plants the roots of which showed the typical colchicine hyperplasia, none of them gave indication of polyploidy.

Various polyploidogenic chemicals. HAVAS obtained in 1937 fasciated shoots (1 in 60) in wheat seedlings treated with morphium and *veratrine* but did not observe at that time their polyploidizing effect. This has since been discovered by GOOS-SÓNNENSCHEIN (1943) and WITKUS and BERGER (1944).

Other agents. Fasciation of the plumule was also observed in one case out of 120 seedlings treated with an extract of *embryonal liver* (kindly furnished by the Hungarian Pharmaceutical Products C° Ltd.). (cf. HAVAS, 1946.)

We wish to emphasize that, although fasciated root-hairs were sometimes observed in untreated plants not a single fasciated root or shoot was found amongst the many thousand control seedlings.

2. TARAXACUM.

Fasciation of the flower stalks and inflorescences.

Taraxacum officinale monstruosities occurring in nature were reported by WEBER (1860). B. GYÖRFFY has obtained such in the tetraploid rubber plant *Taraxacum koksaghyz* (oral communication). They present more than one point of comparison with those to be described.

In the grounds of the Hungarian Biological Research Institute in Tihany a great number of Taraxacum plants were found in 1938 displaying a monstruous fasciation of the flower stalks and of the flowers (Fig. 3). This aberrant form was found only on a narrow stretch 2—3 meters wide and 100 meters long bordering the coast line of the lake Balaton. A thorough search within a radius of about 5 kilometers, both along other places on the lake shore, and in the hilly inland, failed to reveal a single other plant of the same type. Some of these fasciated inflorescences were composed of 5 to 7 single inflorescences tightly squeezed together. The pollen analysis gave no indication of polyploidy.

At the end of the flowering season a number of the aberrant plants as well as of normally flowering plants, provenient from both the lake shore and the hills, were potted. The plants were then divided into five lots. In lot 1 were grouped 20 of the aberrant plants from

the lake shore, in lots 2 and 3 an equal number of normally flowering plants from the same provenience, and in lots 4 and 5, 20 each of the normal plants collected at a distance of about four kilometers at the foot of hills separated by a higher woody ridge from the site where the fasciated flowers were discovered. There was in consequence little probability of cross pollination having taken place between the plants of the two habitats.

Next spring there were only 9 of the experimental plants of the first group, 14 of the second, 13 of the third, 11 of the fourth and 12 of the fifth group which had survived. As soon as the first leaves began to unfold, pieces of cotton wool imbibed with an 1:2000 solution of *colchicine* were placed for 24 hours on the growing tips of the plants of the first, second and fourth groups. This treatment was repeated after 10 days. As soon as the flower buds began to show these were sprayed four times at 4 days' interval with the same concentration of *colchicine*.

As a result of these treatments only one plant of the first group, that is to say of those which were aberrant the year before, produced fasciated inflorescences, whereas as many as 7 were displayed in the second group (Fig. 4) on plants which were previously normal. One of the treated plants of the third group, provenient from the hills, produced also a fasciated inflorescence. None of the untreated controls of groups 3 and 5 was aberrant and moreover, a painstaking search both along the lake shore and in the inland within a radius of about 5 kilometers, a search which was repeated in 1940, 1944 and 1946, failed to reveal another sample of a similar monstruosity.

The pollen analysis of the treated fasciated inflorescences gave some indication of polyploidy, but so did a normal-looking colchicine-treated inflorescence. Some of these inflorescences set fruit but, as is the rule with Taraxacum, the germination of the seeds in artificial conditions was not obtained.

3. TOMATOES.

Fasciation of flowers inducted by acenaphtene.

20 young potted tomato plants of an average height of 30 cms were cut back to a height of 15 cms. Test tubes were rinsed with 0.5 ml of an 1:1000 ether solution of *acenaphtene* until the ether was evaporated and the crystals of the drug were deposited on the walls

of the tubes (KOSTOFF 1938.) These were then slipped over the stumps and stoppered underneath with cotton wool, and the crystals were left to act during four days by sublimation. The total amount of acenaphtene dissolved per tube was 0.0005 gms. but only a fraction of this was actually absorbed by the plants. 18 control plants were cut back as above and empty test tubes previously rinsed with ether were placed after evaporation of the ether, on the stumps. 22 untreated plants served as supplementary controls.

As a result of the treatment the growth in length of the acenaphtene plants was considerably stunted as compared with the controls cut back at the same time (see Table 1.), but in spite of this, the number of the flowers of the acenaphtene plants was considerably larger than of the controls, although on the other hand, relatively few set fruit.

TABLE I.

Date	July 29.			Aug. 17.			Sept. 2.			Sept. 10.			Sept. 26.		
	Height cm.	No. of flowers	No. of fruits	Height cm.	No. of flowers	No. of fruits	Height cm.	No. of flowers	No. of fruits	Height cm.	No. of flowers	No. of fruits	Height cm.	No. of flowers	No. of fruits
Acenaphtene	29.1 ± 2.4697	16	—	32.0 ±2.8230	18	4	47.9 ±5.1058	47	5	55.5 ±5.7647	39	19	64.8 ±6.8823	11	9
Control	31.7 ±2.5882	10	—	47.2 ±3.6235	9	7	69.7 ±4.6823	26	11	77.5 ±5.5058	21	28	90.6 ±7.0564	7	13

Many aberrant leaves were found in the acenaphtene series and one of these displayed the rare form of fasciation known under the name of crateria or ascidia (5a.). Two plants with fasciated flowers were also observed. The pollen analysis of these gave no indication of polyploidy. On the other hand GYÖRFFY has frequently observed fusion of the anther-tubes in polyploid tomatoes obtained by *colchicine* treatment (personal communication). None of the two groups of control plants has displayed fasciated leaves or flowers.

4. BEANS.

Fasciation of leaves.

134 bean plants (Phaseolus vulgaris) were cut back immediately above the primary leaves and pieces of cotton wool moistened with an 2:1000 solution of *colchicine* were placed on the wound surface of the

stumps, and kept there for 24 hours. Polyploidy was elicited as a consequence of this treatment in 60 (44.7%) of the experimental plants. Out of these polyploid plants — and only the polyploid plants — 10 produced aberrant leaves. The average size of the stomata of these was 49.6 ± 3.12, μ as against 32.45 ± 5.08 μ of the controls. The most frequent of these anomalies were the fusion of one of the lateral leaflets with the apical leaflet (Fig. 5a) and "double-peaked" leaflets (Fig. 5b), arising as a consequence of incomplete differentiation.

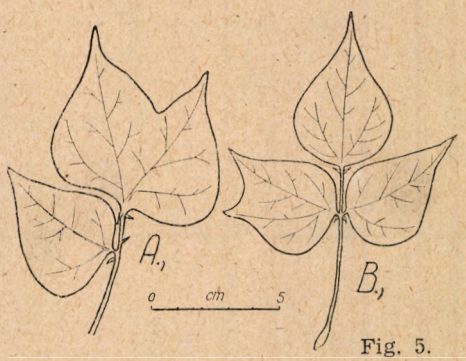

Fig. 5.

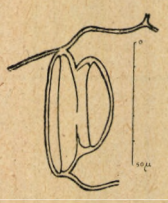

Fig. 6.

Fig. 5. A = fusion of lateral and central leaflet of bean plant. B = "double peaked" leaflet, both colchicine-treated.

Fig. 6. Aberrant, coalescent stomata from a leaf of a colchicine-treated bean plant.

Fig. 6. shows aberrant coalescent stomata from the same colchicine series; similar anomalies were also noted by GAVAUDÁN (1938). Seedlings of bean exposed to the vapours of *acenaphtene* or *naphtalene* also produced aberrant leaves (2 out of 36 plants), i. e. one with a double-peaked leaflet and one with four leaflets. These were not polyploid.

Monophylly was observed in one plant grown from seeds germinated in an emulsion of *benzpyrene*. Out of the 9 plants of the F_1 generation of the above monophyllous plant, double peaked and supernumerary leaflets were found on two.

We wish to stress the point that although several hundred untreated bean plants were grown simultaneously with the above for other purposes and although adequate numbers of controls were grown along with the treated, not a simple case of teratism was noticed amongst the untreated. It seems that although, as is known, constant polyploidy is rarely elicited in beans by polyploidogenic agents the plants respond instead, easily enough, with the production of aberrant forms.

5. PELARGONIUMS.

WINGE (1927), KOSTOFF and KENDALL (1932), KRENKE (1933) and others have described polyploid shoots arising from plants inoculated with *Bacterium (Phytomonas) tumefaciens,* the causative agent of the phytocarcinomata known under the name "crown gall". HAVAS (1942) has obtained a series of entirely tetraploid *Pelargoniums* of the "gigas" type by repeated inoculations with this organism. There is in consequence no doubt about the fact that these bacteria are polyploidogenic agents.

It was mentioned in the above communication (l. c.) that, as a further consequence of this treatment, fasciations in the form of *ascidia* (crateria) have made their appearance (Fig. 7.): the first one in 1936 on a single plant, and then, after an interval of four years, an ever increasing number on an ever increasing number of plants. These teratisms were shown to be tetraploid, the average size of the stomata being 43.54 μ as against 29.16 of the controls, while the number of the chromosomes in the root tips of the aberrant plants was found to be 36, instead of 18 as in the controls. None of the plants which remained diploid after the inoculations have displayed ascidia. Neither are other forms of fasciation, obtained in other plants inoculated with *B. tumefaciens (Smith 1922,* BROWN 1927.) or the parent plants themselves, known to be polyploid. Yet, as in tomato treated with acenaphtene, a leaf transformed in ascidia was obtained in radish treated with *thrombine,* without being accompanied by chromosome duplication.

DISCUSSION.

In endeavouring to explain the mechanism of the different forms of fasciation described it seems necessary to recur to different interpretations according to the organs affected, although connecting links are not lacking for the construction of a more synthetic theory of the phenomena observed.

As regards the fasciation of roots, for instance, the explanation given for the fusion or coalescence of tree trunks or branches may be adapted, but not adopted. According to this theory (MÁNDY 1940.) the mechanical injury caused by the wind rubbing young stems or branches one against the other may effectuate, by the stimulation of wound callus formation and subsequent healing, a sort of "grafting by approach" at the surface of contact. In the experimental conditions used it may be suggested that instead of the mechanical injury a "phy-

sico-chemical" injury, caused by the colchicine treatment, was the "primum movens". The circumstance that as a consequence of tumefaction and of the hyper-hydration of the tissues, caused by colchicine, fissures were often observed in the epidermal layers, as well as suberization and callus formation, speaks in favour of this interpretation. The fusion of the roots which have come to grow accidentally in contact with each other in the measured and protected space of the Petri dishes, is thus more plausibly explained than is the fusion of trees and branches in the woods, where after a windy period a long period of complete calm has to be supposed in order that the trees or branches in contact should not be, again and again, torn asunder.

Similar conditions may be supposed to have been *one* of the causes of the fasciation of shoots found in wheat seedlings, as confirmed both by their gross morphological picture and by their histological analysis, revealing the fusion of the vascular elements.

One cannot help being struck, however, by the examples given of the endemic, serial emergence of the teratisms observed in *Taraxacum* and *Pelargonium,* in both space and time. We can add to these the wholesale appearance of fasciated trees in the oak forests of Felsőgalla (Hungary), where about 30 were found within a radius of 3—400 meters while not a single one was discovered many kilometers around.

In view of such phenomena one is led to think of the "periods of mutation" of DE VRIES (1901) and of the evolutionary significance of similar coincidences of "inner disposition" and environmental stimuli, which, through a series of "trials and hits" sensitizations and micromutations may lead to mutation proper.

The theory of WHITE (1916) according to which "a character is just as much an effect of a specific environmental medium as it is an effect of a bit of protoplasmic material" rather confirms than infirms this view.

Whether experimentally induced or occurring in nature, the examples given also recall the lesson taught by LUTHER BURBANK who repeatedly emphasized that: plants with the greatest variability are those which offer the greatest chance of success to the plant breeder. Plants which respond readily to polyploidizing or other agents with the production of teratisms belong undoubtedly to this category - whether, as we have seen, they induce chromosome duplications or not. By taking up their systematic experimental investigation colchicine research would serve well the creative spirit of BURBANK the "architect of nature".

SUMMARY.

Examples were given of fasciation and kindred teratism obtained in different plants by the administration of polyploidizing and some other agents. It was shown that these phenomena, although mostly connected with the oncogenic and polyploidizing actions of the substances used, are not exclusively conditioned by them. It was also demonstrated that the responses elicited are in a great measure independent of the chemical constitution of the agents used. Attention was drawn to the endemic, serial emergence of the teratism observed, to their evolutionary significance, and to the possible importance of induced teratisms from the point of view of plant breeding.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for help in the English translation.

REFERENCES.

1. ALDROVANDI U. (1642), *Monstrorum Historia. Bononiae. Typis Nicolai Tebaldini*
2. BROWN N. A. (1927), *Phytopathology* **17.**: 141.
3. CHOUARD P. (1938), *Bull. Soc. Bot. France* **85.**: 546.
4. DOBZHANSKY Th. (1939), *Die genetische Grundlagen der Artbildung. Jena.*
5. DUSTIN A. P.—Havas L. J.—Lits F. (1937), *C. Assoc. Anat. Marseilles.*
5a. GALLE L. (1942), *Bot. Közl.* **37.**: 66.
5b. GAVAUDAN P. et N. (1938), *C. R. Soc. Biol. Paris.* **128.**: 714.
6. GEORGESCU C. (1926), *Mitt. deutsch. Dendrol. Ges.* **2.** no 37.
7. GOOS-SONNENSCHEIN Cl. (1943), *Züchter* **62.** (3): 171.
8. HAVAS L. J. (1936), Comm. IInd *Internat. Cancer Congr.* 436. Bruxelles.
9. HAVAS (1937a), *Nature (Lond.)* **143.**: 371.
10. HAVAS (1937b), *Bull. Assoc. France d. Cancer.* **26.**: 1.
11. HAVAS 1938), *Growth* **2.** (3): 257.
12. HAVAS (1940), *Conf. Soc. Roy. Sci. Med. et Nat. Bruxelles 1er Janv. 1940.*
13. HAVAS (1942a), *Bull. Acad. Roy. Belgique. Cl. Sci. V. ser.* **28.**: 318.
14. HAVAS (1942b), *Tumori,* A **38.** (3—4): 163.
15. HAVAS (1946), *Nature* (Lond.) **157.**: 726.
16. HAVAS *(in press): Bull. Agric. Univ. Fac. of Hort. Budapest.*
17. HAVAS and —Mendeleeff P. (1937), *C. R. Soc. Biol.* **126.**: 83.
18. KLEBS G. (1903), *Willkürliche Entwicklungsänderungen bei Pflanzen. Jena I:* **89.**
19. KOSTOFF D. (1933), *Arch. Microbiol.* **4.**: 135.
20. KOSTOFF (1938), *Curr. Sci.* **7.**: 108.
21. KOSTOFF and —Kendall J. (1932), *Science N. S.* **76:** 150.
22. KRENKE N. P. (1933), *Wundkompensation, Transplantation und Chimaeren bei Pflanzen.* Berlin.
23. KRUG C. A.—MENDES A. J. T. (1942), *Rev. Agric. Piracicoba* **18.**: 393.

24. KÜSTER E. (1925), *Pathologische Pflanzenanatomie* **3.** *Aufl. Jena.*
25. LEVAN A. (1945), *Arch. J. Claus Stift.* **20.:** 142.
26. LIESKE P.(1928), *Cbl. f. Bakt. Paras. Infektionskrankh. I:* 8 and *II:* 76.
27. MÁNDY Gy. (1940), *Term. Tud. Közl.* **72.:** 334.
28. MAGROU J. (193), *Ann. d. Sc. Nat. Botanique Ser.* **10.:** 17.
29. MOQUIN-TANDON A. (1841), *Elémcnts de teratologie végétale. Paris.*
30. MÜNTZING A. (1936), *Hered.* (Lund) **21.:** 263.
31. de QUERVAIN F. (1913), *Über die Stellung der Geschwülste unter den Naturerscheinungen. Vortr. Naturf. Ges. Basel. Leipzig.*
32. RANDALL T. E.—RICK C. M. (1945), *Amer. J. Bot.* **32.:** 560.
33. RIKER A.—RILDEBRAND E. M. (1943), *J. Agric. Res.* **48.:** 60.
34. RYBIN V. A. (1939), C. R. (Doklady) *Acad. Sci. URSS.* **24.** 4: 368.
35. SCHLOTTERBECK P. J. (1755), *Schediasma botanicum de Monstris Plantarum quo analogiam, regno vegetabili cum animali intercedentem, in producendis iisdem, adstruit Acta Helvetica Phys.-Math.-Both.-Med.* **2.** *Basilea p. 1—14.*
36. SMITH E. F. (1922), *Phytopathology* **12.:** 363.
37. STROHL J. (1929), *Missbildungen in Tier und Pflanzenreich. Jena.*
38. de VRIES H. (1901), *Die Mutationstheorie, Versuche und Beobahctungen über die Entstehung der Arten. I—II Leipzig.*
39. WEBER N. (1860), *Verh. Nat. Ver. preuss. Rheinl. u. Wes'ph.* **17.:** 20.
58a. WHITE O. E. **(1916).** *Zschr. f. ind. Abst. Vererb.* **16.:** 49—
40. WINGE O. (1927), *Z. Zellforsch.* **6.:** 397.
41. WINGE O. (1930), *ibid.* **10.:** 638.
42. WITKUS E. R.—BERGER C. A. (1944), *J. Hered.* **35.:** 131.
43. WORSDELL W. C. (1915—16), *The Principles of Plant-Teratology* 2 vols. *London.*

EXPLANATION OF FIGURES IN THE TABLE.

Fig. 1. Colchicine-treated wheat seedling with fasciated tumefied roots fusioned at the proximal end and divided at the distal end. The basis of the shoot is also monstrously tumefied.

Fig. 2. Colchicine-treated wheat seedling with fasciated tumefied roots divided at the proximal end and fusioned at the distal end. The plumule is thickened but not tumefied.

Fig. 3. Right: fasciated **Taraxacum** inflorence and flower stalk found in nature, center: normal inflorescence; left: fasciated flower stalk split and rolled up naturally as an effect of turgor pressure and the unequal growth of the exterior and interior epidermal tissues.

Fig 4. Right and left colchicine-treated **Taraxacum** inflorescences, in the center a control inflorescence.

Fig. 7. Crateria (ascidia) from a colchicine-treated tetraploid Pelargonium

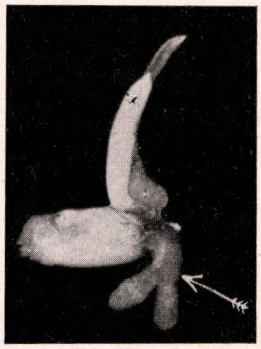

Fig. 1.



Fig. 2.



Fig. 7.



Fig. 3.



Fig. 4.

CURATIVE EFFECTS OF DARKENING STEM TIPS UPON PHYTOCARCINOMATA.

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With 1 Figure and 1 Table in the text.

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INTRODUCTION.

A parallel was drawn, in previous communications, by Havas, (1937), between the effects of colchicine, palmitic acid, the removal of flowers and the capping of stem tips upon the development of induced plant tumours (phytocarcinomata). It was shown that all these heterogenous types of treatment produced tumour reduction to approximately the same extent. It was inferred that the fundamental cause of the inhibition thus obtained was a similar, presumably hormonal, mechanism of action. The experiments having since been repeated and extended, the object of the present paper is to report new observations and additional data on the influence of short day conditions induced by capping the stem tips, upon the growth of plant tumours and upon the hormonal syndroms accompanying this effect.

MATERIAL AND METHODS.

Experiment 1. 20 equally developed tomato plants (Lycopersicum esculentum, Mill., var. Kondyne red), grown from seed, were inoculated when about 30 cm. high with a very virulent strain (Stapp II, from Baarn) of Bacterium tumefaciens by means of five equidistant pricks along the main stem of each plant. The plants were then divided into two sets of ten, one of which was reserved for control and the other treated as follows: the upper 10 cms. of the main stem were covered with black cloth caps, following the method described by Zimmerman and Hitchcock (1929). During the first period of treatment (March

17th to April 1st, 1937) the caps were kept on the plants from noon to 6 p. m., during the second period (April 2nd to April 15th) from noon to 6. p. m., and during the last period (April 30th to May 14th) alternately, one day from 9. a. m. to 5 p. m. and the other day from noon to 6 p. m.

Experiment 2. This was an exact repetition of Experiment 1, except that it was carried out in mid-summer (June 11th to Aug. 18th) instead of early spring. In both experiments when about 20% of the tumours showed necrosis they were cut off and weighed separately.

Experiment 3. This experiment was carried out from Aug. 14th to Nov. 15th. Its object was to ascertain whether the same hormonal effects could be elicited in *uninoculated* plants, of different variety and under different seasonal conditions, as in the course of the two preceding experiments. The upper third of the main stem of 30 tomato plants (var. President Garfield) was covered with conical caps made of black paper supported by stakes inserted in the soil. 20 untreated plants served as controls. The plants were chosen at random from cuttings of unequal strength and height (av. height 24 cm., max. 34, min. 18 cm.) many of which showed flowerbuds in the initial stage already at the beginning of the experiment. The caps were left on the treated plants during approximately the same periods and during the same number of hours as above, except that during the last 26 days of the experiment the caps were entirely taken off.

All three experiments were conducted on the greenhouse bench but not in controlled conditions. The plants were re-potted once during the treatment in rich soil and were watered as needed with a fertilizer.

OBSERVATIONS.

Experiment 1.

The tumours began to show eight days after the inoculations. Fig. 1. shows the number of tumours developed and the relative increase of their surface area from the 15th day on, until the end of the experiment.

As is shown by the coefficient of the two largest diameters of the tumours, the growth of the surface *area* of the tumours of the treated plants has caught up and has even surpassed that of the controls. Table 1. shows, on the other hand, that the increase of the surface area corresponded by no means with the increase in *weight* of the tumours, the weight of the tumours of the capped plants attaining barely the half of that of the controls.

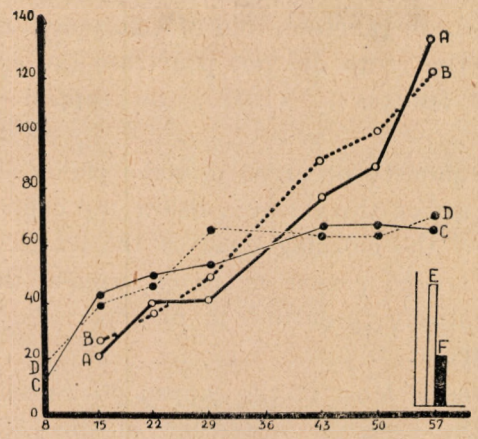

Fig. 1. Surface area, number and weight of tumors. — Coordinata: number and surface area of tumours; abscissa: number of days. — A: surface area of treated tumours; — B: surface area of control tumours. — C: number of treated tumours; — D: number of control tumours. — E: avarage weight of 1 tumour (control); — F: avarage weight of 1 tumour (treated), both in centigrams. — The author is indebted to *Dr. L. J. M. Felföldy* for drawing the figure and for making the statsitical calculations.

The phenomena accompanying tumour reduction in the capped plants were the following:

1. *Roots:* It was noticed on the 15th day of the experiment, when repotting the plants, that the roots of the darkened plants were thicker and more brittle than the roots of the controls. This thickening kept up until the end of the experiment and finds its expression in the increased weight of the roots (Table I.).

2. *Stems:* A marked thickening of the stems was manifest from the 19th day on, and lasted until the end of the experiment. The weight of the stems per. cm. length (Table I.) gives an idea of the extent of this swelling.

3. *Etiolation:* Although the darkened parts of the capped plants remained etiolated until the end of the experiment, this was less marked during the two last than during the two first periods of the treatment.

The longer hours of insolation and the growing intensity of light, as spring progressed, were probably responsible for the diminution of this effect.

4. *Adventitious roots:* Although root primordia were prominent, forming rows of protuberances devoid of chlorophyll all along the upper part of the stem, the roots did not penetrate the epidermis, as found by HITCHCOCK and ZIMMERNAN (1939), in capped but uninoculated tomatoes.

5. *Epinasty:* Epinasty occurred in some plants at the beginning of the treatment in two or three leaves below the capped region, towards the evening, but by the morning most of the leaves had recovered. In the course of the second period of the experiment the leaves appeared to have become entirely insensible to photoperiodic stimulation and epinasty did not recur.

6. *Axillary shoots:* Most of the capped plants had a more "stocky" appearance than the controls on account of the larger number and the increased length of the axillary shoots.

7. *Flowers and fruits:* Although the experiment was not carried to the end of the flowering and fruiting phases, there is indication of a slight increase in the number of flowers in the capped plants and of a decrease in the number of fruits. On the other hand, the average weight of the individual fruits was markedly superior to that of the control fruits, (see Table I.).

Experiment 2. The results obtained in the course of this experiment confirm on the whole those outlined above, although the differences between the capped and the control plants were much less accentuated than in the first experiment as regards the respective weight of the tumours. It is also to be noted that the absolute weight of the tumours was much inferior to that previously recorded (see Table II). On the other hand the accompanying transverse growth responses of the capped plants were quite as marked as those observed in the course of the first experiment, with the exception of epinasty which was hardly noticed, even at the beginning of the experiment. The failure to produce this reaction was no doubt due, like the inferior development of the tumours, to seasonal differences as well as to the fact that, while in the course of the first experiment the atmosphere of the greenhouse was highly saturated with humidity, it was very dry in the course of the second experiment. The tendency towards the swelling of the fruits was, however, again confirmed.

TABLE I. TABLE II. TABLE III.

No. of Experiment Date of experiment	III/18—V/14 1.		VI/11—VIII/18 2.		VIII/14—XI/15 3.	
Treatment	*Capped*	*Control*	*Capped*	*Control*	*Capped*	*Control*
No. of plants	10.	10.	10.	10.	30.	20
No. of tumours per plant.	6.5 ± 0.21	6.8 ± 0.20	4.6 ± 0.22	4.3 ± 0.23	==	==
Av. surface area of 1 tumour.	132.4 ± 9.63	121.3 ± 6.67	104.8 ± 3.95	110.5 ± 4.39	==	==
Av. weight of one tumour, mgms.	208.8 ± 0.01	447.9 ± 0.03	168.0 ± 0.10	230.0 ± 0.08	==	==
Av. height of plants, cms.	86.6 ± 2.91	90.0 ± 2.83	91.5 ±10.06	101.0 ± 5.54	81.1 ± 2.09	90.1 ± 3.21
Av. weight of stem & leaves p. plant, g.	79.7 ± 4.18	63.4 ± 4.21	93.0 ± 4.92	90.8 ± 4.54	127.0 ± 3.73	125.4 ± 3.98
Av. weight of stem alone p. pl. gms.	32.0 ± 1.15	26.1 ± 1.28	34.2 ± 2.56	32.3 ± 1.86	39.4 ± 1.27	35.1 ± 1.06
Av. weight of roots p. plant, gms.	23.6 ± 0.79	17.6 ± 0.83	25.9 ± 1.51	15.7 ± 0.78	36.4 ± 1.05	24.9 ± 0.92
Number of flowers p. plant.	4.1 ± 0.37	3.5 ± 0.29	7.5 ± 0.58	6.3 ± 0.70	==	==
Av. number of fruits p. plant.	1.4 ± 0.08	1.6 ± 0.06	3.6 ± 0.29	4.1 ± 0.26	5.6 ± 0.32	6.7 ± 0.35
Av. weight of fruits p. plant, gms.	7.0 ± 0.96	5.8 ± 0.76	79.9 ± 3.45	68.3 ± 4.05	230.5 ± 6.58	219.7 ± 7.63
Av. weight of one fruit, gms.	5.5 ± 0.58	3.7 ± 0.07	23.2 ± 1.56	17.0 ± 0.55	37.7 ± 4.19	33.93 ± 1.61

Expriment 3. This experiment was carried on until the ripening of the fruits and lasted, in consequence, very much longer than the two preceding experiments. Yet, as Table III shows, the transverse growth effects lasted in the capped plants until the end of the experiment and this, in spite of the fact that the caps were taken entirely off during the last 26 days of the experiment. Moreover, in some plants the adventitious roots, which in the inoculated plants remained in an initial stage, broke through the epidermis and the proliferation of the axillary shoots was also more accentuated than in the preceding experiments. A considerable thickening of the nodes of the capped plants was also noticed, their average diameter attaining 15 mms. as against 9.5 mms of the controls.

DISCUSSION.

The interpretation of the above findings is difficult as regards the tumour reductions obtained. The experimental results were, indeed, exactly the opposite of what could have been expected according to our working hypothesis.

This hypothesis was founded upon the transverse reactions of plants to photoperiodic interventions which, according to KNOTT (1936), GARNER & ALLARD (1924), ZIMMERMAN & HITCHCOCK (1936), HITCHCOCK & ZIMMERMAN (1939), may cause swellings, epinasty, adventitious root formation, proliferations, etc. on the stems of the treated plants.

It was expected that the loss of the apical dominance and the transverse transport of the endogenous phytohormones, elicited by excluding light from the terminal buds, would also increase the supply of auxins for the phytocarcinomata located along the stem, and that a stimulation of their growth would ensue, a stimulation similar to that observed e. g. in the lateral buds.

Perplexity about empirical facts as contrary to theory as in the above findings is heightened by the circumstance that the *bacteria* introduced by the inoculations *elaborate a growth hormone themselves* (BROWN & GARDNER, 1936, BERTHELOT & AMOUREUX, 1937, GIOELLI, 1940, HAVAS, 1942, and that, in consequence, rather than inhibition, cumulative effects could reasonably have been expected.

But it is perhaps precisely by examining the possible part played by this additional suply of hormones that the above tumour reductions may become intelligible. If, in fact, the theory of THIMANN (1937) holds true and the development of the lateral buds depends upon an optimum concentration of auxins, under and above which there is little or no growth, then, the inhibition of tumour development by a suply of growth substances exceeding this optimum can also be understood.

On the other hand we must also take into consideration the notion of bud "inhibition", as understood by BORGSTRÖM (1939), according to whom this inhibition consists of two different phenomena: one of which is the failure of the buds to grow out, being short of hormones, and the other which consists in an increased radial extension of bud tissue under the influence of an increased radial distribution of the hormone. If the comparison between the potentialities of lateral development and lateral tumour development is not too far fetched, it seems to us that the increased radial growth of the tumours of the capped plants, as expressed by their surface coefficient, (see Tables I and II), at the expense of their protuberance (swelling), as expressed by their *weight* (see Tables I and II), is an indication of a mechanism of action similar to the one which governs bud inhibition, according to BORGSTRÖM. A comparison between the relative surface area coefficient and the weight of the control tumours as well as the fact that in the inoculated plants the adventitious roots failed to grow out, as opposed

to the uninoculated plants (exp. 3.), seem to strengthen this hypothesis. How far the additional supply of growth substances furnished by the bacteria contributes to these phenomena by increasing the permeability of the phloem and enabling "an increased lateral passage of auxins" (BORGSTRÖM, l. c. 131) is one more of the questions which we must leave open.

We stand on a firmer ground as regards the interpretation of the hormonal growth effects observed in the capped plants. The phenomena described above in detail as accompanying tumour reduction in the capped plants are in fact by no means specific. They are typical transverse reactions which confirm in all essentials the findings of ZIMMERMAN and HITCHCOCK (1936) and HITCHCOCK and ZIMMERMAN (1939) in capped but uninoculated plants. Our own findings in capped but uninoculated plants (exp. 3) tend to demonstrate also that the bacterial growth substances had no discernable part in the intensity of the transverse responses except in so far as the outgrowth of the adventitious roots on the stem seemed to be inhibited by them. There is only one observation to be added to those of the eminent workers of the Boyce Thompson Institute and this is, that although inferior in number the average weight of the individual fruits had a tendency to exceed that of the controls, (see Tables). This also seems to be a lateral growth effect which has been observed under the influence of the most varied and chemically unrelated substances (Havas, 1937), in both tumour bearing and uninoculated plants.

SUMMARY.

A considerable reduction of the weight of induced phytocarcinomata was obtained in tomato plants inoculated with Bacterium tumefaciens, by capping the stem tips with black cloth or paper. The hormonal syndroms accompanying this effect, such as: epinasty, swelling of the stem and of the roots, stimulation of the initiation and development of the axillary shoots. etc. were interpreted as typical transverse reactions, and the tumour reductions obtained were tentatively explained by a similar mechanism of action.

Grateful acknowledgment is made to Mrs.. J. THOMPSON VASS for help in the English translation.

LITERATURE CITED.

1. Berthelot A. and Amoureux G.: C. R. Acad. Sci. Paris, **205,** 476 ,(1937).
2. Borgström G.: The Transverse Reactions of Plants, Gleerup, Lund, (1939).
3. Brown N. A. and Gardner T. E.: Phytopath. **26,** 7, 708, (1936).
4. Garner W. W. and Allard H. A.: J. Agr. Res. **23,** 871, (1923).
5. Gioelli F.: Riv. Pat. Veget. **30,** 3/4, & **30,** 9/10, 1, (1940).
6. Havas L. J.: Bull. Assoc. Fr. d. Cancer, **26,** 6, 1, (1937).
7. Havas L. J.: Nature, CXV, 191, (1937a).
8. Havas L. J.: Tumori, XXVIII, fasc. III & IV, 163, (1942).
9. Havas L. J.: Bull. Acad. Roy. Belgique, 30, (1942).
10. Hitchcock A. E. and Zimmerman P. W.: Contrib. Boyce Thompson Inst. **10** 389, (1939).
11. Knott J. E.: Contrib. Boyce Thompson Inst. **8,** 311, (1936).
12. Thimann K. V.: Am. J. Bot. **24,** 407, (1937).
13. Zimmerman P. and Hitchcock A.: Contr. Boyce Thompson Inst, **1,** 467, (1929)
14. — — — —: — — — — **8,** 311, (1936).

L'ACTION DE LA COLCHICINE, ADMINISTRÉE SEULE OU EN COMBINAISON AVEC DES HORMONES, SUR LA CROISSANCE ET SUR LE DEVELOPPEMENT DES EMBRYONS DE LA GRENOUILLE.[1]

(Communication préliminaire).

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Deux graphiques et un tapleau dans le texte.

(Reçu: Janvier 1942.)

Dès les premières applications de la colchicine à l'étude des stimulations hormonales (F. J. Lits, 1936, E. Allen, & al. 1937) le trait d'union qui reliait les investigations des chercheurs fût l'utilisation de l'alcaloide à titre de "détécteur" de la sensibilisation des cellules à la division, telle qu'elle est exercée par certaines hormones animales endogènes ou expérimentalement administrées.

Nous avons toutefois pensé -et nous avons pu confirmer cette hypothèse en provoquant p. e. des mouvements nastiques chez les plantes, la stimulation de la croissance, de la floraison et de la guérison des blessures, ainsi que l'apparition d'organes adventifs (L. Havas 1937a, 1937b, 1938, 1939a, 1940c), ou encore, en déclanchant la "robe nuptiale" chez des poissons (Rhodeus) ou en stimulant la croissance des testicules chez les rats (Havas 1939a, b, 1940a, b, c) —: que *le rôle de la colchicine pouvait dépasser celui d'un simple "enregistreur de mitoses"*. Nous avons pensé, en effet, que cette substance pouvait déclancher *elle*

[1] Ces expériences furent entreprises le 16 Mars 1939 en collaboration avec le Professeur J. Méhes de l'Université de Kolozsvár. Les circonstances l'ayant empêché de terminer la part de travail qui lui incombait j'ai le regret de devoir me charger seul de la publication des résultats, auxquels il a d'ailleurs aussi contribué ce dont je le remercie.

même des actions "hormonales" ou hormono-mimétiques[2], soit directement, soit en agissant sur les organes endocrins.

Ayant relevé, en outre, des synergismes et des antagonismes d'action de la colchicine avec des hormones endogènes ou expérimentalement administrées, l'extension de ces recheches au test classique de *Gudernatsch* (1912, 1914) nous parut motivée. Nous avons voulu examiner, en effet, si, et en quelle mesure la colchicine pouvait influer, administrée seule[1] ou en combinaison avec les hormones du thymus et de la thyroide, sur la croissance et sur le développement des têtards de la grenouille.

Matériel et technique.

Nous nous sommes servis d'embryons de Rana fusca, recueillis au premier stade du bourgeon caudal le 14 et 22 Mars 1939. Les larves furent élevées dans l'eau de conduite à laquelle les substances suivantes furent ajoutées pendant des périodes de durée plus ou moins longue: colchicine (Merck), thyroxine synthétique cristallisée (Henning), Thyreosan (Extractum thymi de Richter).

Exposé des résultats.[2]

*I*er *groupe: Influence de la colchicine administrée seule.*

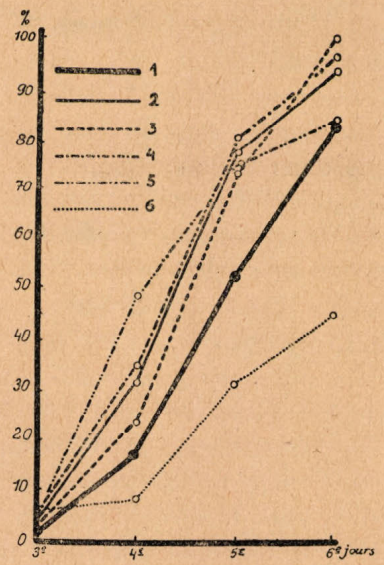
Nombre total des embryons employés: 2796. Durée des expériences: 34, 29, 18, 13, 7 et 50 jours.

Le graphique no. I montre l'influence des diverses concentrations de la colchicine sur le rythme de *l'éclosion* des larves et le tableau no. 1. leur influence sur leur *croissance* en longueur. Notons en outre que dans la concentration de 1:10,000 des *périodes* de stimulation de la croissance pouvaient aussi être remarquées et que dans les concentrations de 1:100,000 et 1:200,000 cette stimulation s'est maintenue pen-

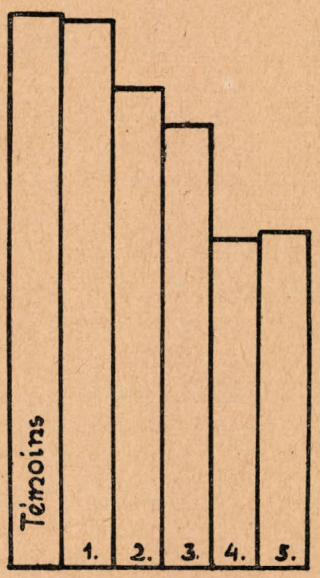
[2] Voir pour des confirmations ultrérieures des actions „hormonales" de la colchicine: R. Delcourt 1938, 1939, J. Dragoin et C. Crisan 1939, C. Laur 1938, 1939, A. Levan 1942, J. Soyano 1940.

[1] Voir pour l'influence de la colchicine, administrée seule, Laur 1938, 1939, Bushnell 1938, Keppel et Dawson 1939, Delcourt 1938, 1939, Wolsky 1940, et Allodiatoris 1941.

[2] L'article ayant dû être réduit au quart de la longueur originale nous avons du ramener à deux les expériences exécutées en six séries, de durées différentes. Un compte rendu plus détaillé est réservé pour l'avenir.



Graphique No. I: % des éclosions. 1. Témoins, 2. Colch. 1:10.000, 3. colch. 1:7500, 4. colch. 1:5000, 5. colch. 1:2500, 6. Colch. 1:1000.



Graphique No. II. Longeur des larves (mm). Témoins (11.25±0.29) — 1. Colch. 1:10.000 (11.11±0.34) — 2. Colch. 1:7500 (10.0±0.90) — 3. Colch. 1:5000 (9.0±0.32) — 4. Colch. 1.2500 (6.88±0.56) — 5. Colch. 1:1000 (7.00±0.46).

dant toute la durée du traitement (tableau no. 1.). Pourtant au cours de nos expériences ni le rhytme de l'éclosion, ni la stimulation de la croissance n'a atteint les proportions dont LAUR (1938, 1939) a rendu compte. Dans les concentrations de 1:10,000 et 1:20,000 la colchicine a aussi stimulé la *différenciation* des têtards au commencement de la métamorphose tandis que dans la concentration de 1:100,000 une légère avance fût maintenue jusqu'à la fin de l'expérience (tableau no. 1.).

Les *anomalies morphologiques* suivantes furent remarquées chez les larves dans les solutions de colchicine de 1:1000 et 1:5000: boursouflures oedematiques, tuméfaction des bourgeons caudales et des bourgeons des appareils olfactif et branchial. Chez la plupart de ces animaux on remarquait aussi de la microcephalie (voir aussi KEPPEL et DAWSON l. c. et WOLSKY l. c.) et chez plusieurs aussi de la cyclopie, ainsi que des queus hélicoïdes rappelant celles des têtards parthénogéniques de KAWAMURA (1939). En outre quoique ne provoquant sur cet organe pas de phénomènes proprement dits tératologiques, la colchicine occasionne cependant la cessation du fonctionnement de l'appareil adhésif sous-buccal.

IIe groupe: Traitement combiné des têtards avec de la colchicine et des horomones, après l'éclosion.

Nombre total des embryons traités: 1145. Durée des expériences: 13, 7 et 50 jours.

Le tableau no. 1 offre un exemple des effets obtenus avec les divers traitements combinés. En résumant ce relevé et les résultats enregistrés au cours des autres expériences de ce groupe on peut constater ce qui suit:

a) Ajoutée à la *thyroxine* (1:200,080, 1:300,000, 1:400,000) et à l'extrait thyroidien *"Thyreosan"* (1:150, 1:200) la colchicine (1:100,000, 1:200,000) inhibait en général, ou du moins pendant certaines périodes du traitement, la croissance en longueur des larves et stimulait, au contraire la différenciation de leurs organes (métamorphose), comparativement aux témoins et comparativement aussi aux larves traitées avec la colchicine seule.

b) Ajoutée à l'extrait de *thymus* (1:150, 1:200) la colchicine (1:100,000, 1:200,000) inhibait la croissance en longueur, comparativement à l'extrait de thymus seul et comparativement aux témoins et pendant certaines périodes du traitement stimulait la différenciation. Ces effets furent cependant moins marqués que ceux des traitements combinés avec la thyroxine et la thyreosan.

Il est à noter, en outre, que les hormones ajoutées à la colchicine en augmentent la *toxicité*. Ceci se manifesta, avant tout, par l'augmentation de ses effets léthaux et puis par l'exaspériation des réactions nerveuses pathologiques, telles que la léthargie, les convulsions et les mouvements natatoires en manège. La *pigmentation* des animaux traités avec la colchicine additionnée de thyroxine était plus foncée que celle des animaux traités avec la thyroxine ou la colchicine seules, ou encore que celle des témoins non traités.

TABLEAU I.*

Traitement	Degré de différenciation	Longuere moyenne (mm).
Témoins eau)	+ + + + +	34.5 ± 0.63
Colchicine 1:100,000	+ + + + + +	36.1 ± 1.03
„ 1:200,000	+ + + + + +	36.7 ± 0.31
Thyroxine + colchicine 1:100,000	+ + + + + + + + +	21.2 ± 1.04
„ + „ 1:200,000	+ + + + + + + + + + +	20.0 ± 0.55
„ seule 1:400,000	+ + + + + + +	24.1 ± 0.98
Thyreosan + colchicine 1:100,000	+ + + + + + + + +	20.0 ± 0.85
„ + „ 1:200,000	+ + + + + + + +	21.2 ± 0.94
„ seule 1:200	+ + + + + + + +	23.6 ± 1.15
Thymus + colchicine 1:100.000	+ + + +	33.0 ± 0.79
„ + „ 1:200,000	+ + + + + +	32.1 ± 0.79
„ seul 1:200	+ + +	40.5 ± 0.97

Considérations générales.

En posant une fois de plus la question des possibilités et des limites de l'utilisation de la colchicine pour l'étude des problèmes d'endocrinologie, l'examen des liens et des divergences entre ses actions cytologiques, pharmacodynamiques et "hormonales" s'impose.

Nous avons vu, qu'administrée seule, la colchicine pouvait déclancher certaines actions "hormonales", ou hormono-mimétiques, semblables à celles provoquées, selon GUDERNATSCH (l. c.), par la thyroxine ou des extraits thyroidiens, et que les effets des deux substances mélangées pouvaient s'additionner. Ajoutée au thymus, la colchicine avait, au contraire la tendance d'en contrarier les effets classiques sur la croissance et sur la différenciation des organes (métamorphose) des têtards. Par conséquant, l'emploi de la colchicine en tant que révélateur des actions

* Je remercie le Docteur L. Felföldy pour son aide dans les calculs statistiques.

mitotiques de la thyroxine et du thymus nous parait sujet à caution, que ces hormones soient endogènes ou expérimentalement administrées.

En recherchant les causes des actions "hormonales" de la colchicine et les liens qui les relient à ses actions pharmacodynamiques, il n'est pas sans intérêt de noter:

1) l'inhibition du développement du cerveau des têtards colchicinés (Wolsky, l. c.), car il est difficile de s'imaginer que des lésions pareilles puissent rester sans répercussion sur l'hypophyse et, par conséquant, sur les phénomènes connexes de la croissance et de la métamorphose;

2) les troubles de glyco-régulation produits, d'une part par la colchicine (L. Arvy 1940), d'autrepart par la thyroxine (St. Bilevicz 1938), puisque la capacité des têtards à parfaire leur métamorphose dépend de leur glyco-régulation normale (Bilevicz, l. c.);

3) les "réactions d'alarme" colchiciniques (C. P. Leblond 1939), et notamment, l'involution du thymus et l'hyperplasie du cortex, dont les répercussions thyroidiennes sont connues et qui peuvent, par conséquant, également influer sur la métamorphose. Comme dans le cas de la glyco-régulation, la signification de ces observations est doublée par l'action parallèle de la thyroxine qui provoque aussi l'hyperplasie surrénale, selon Ingle et Higgins (voir Leblond l. c.);

4) les effets nerveux de la colchicine (convulsions, mouvements en "manège" etc.) que nous avons mentionné et dont nous trouvons aussi les parallèles dans les actions de la thyroxine (E. Herzfeld et A. Frieder 1933 et A. Lánczos 1939).

Ces parallèlismes d'action de la colchicine et des hormones employées font doublement ressortir la difficulté de l'interprétation exacte des images cytologiques obtenues et nous paraissent motiver la nécessité de la revision de la technique colchicinique pour l'évaluation des stimulations mitotiques d'origine hormonale.

RESUME.

Il a été constaté qu'administrée aux embryons de la grenouille, la colchicine provoque des actions dynamiques et des anomalies morphologiques et que dans les concentrations appropriées elle inhibe la croissance en longueur des larves, sauf dans les concentratiors de 1:100,000 et 1:200.000, tout en stimulant la différenciation de leurs organes (métamorphose). Des actions dynamiques et morphologiques de la thyroxine, (et, à un moindre degré, de la thyréosane), semblables a celles de la colchicine, ont aussi été relevées.

Il a été observé qu'ajoutée à la thyroxine la colchicine détermine une exaspération d'une série des réactions dynamiques et morphologiques tandis que combinée avec un extrait du thymus la drogue a la tendance d'en contrarier les effets caractéristiques.

Il a été conclu, qu'étant donné les syndromes hormono-mimétiques de certaines actions de la colchicine et ses synergismes et antagonismes d'action avec les hormones administrées, son utilisation, en tant que "détecteur" des stimulations hormonales, est sujet à caution.

REFERENCES.

REVUES.

1. ARVY, L. (1940), Rev. Scientifique, **3**, 153—164.
2. CAVALLERO, C., (1939), Tumori, XXV. 506—528.
3. CHODKOWSKY, K., (1937), Protoplasma, XXVIII.—597—619.
4. DUSTIN P., (1939), Arch. Exper. Zellforsch. **22**, 395—406.
5. FYFE, J. L., (139), Imp. Bur. Plant Ind., 576.
6. GYÖRFFY, B., (1939), Acta. Univ. Szeged, **5**, I—29.
7. HAVAS, L., (1939a), Chem. Prod. **2**, 61—65.
8. Id. (1940a), J. Heredity, **31**, 115—117.
9. Id., (1940c), Soc. Roy. Sci. Méd. & Nat. Bruxelles, Janvier, I—30.
10. KOSTOFF, D., (1941), Centr. Agr. Res. Inst. I—85.
11. LITS, F. J., (1936), Arch. Internat. Méd. Exper. **11,—12,** 901.
12. LEBLOND, C. P., (1939), Ann. Endocrinologie, *I*, 180—196.
13. MARINE, D., (1935), Gland. Physiol. & Therapy, 315—333.

COMMUNICATIONS.

14. ALLEN E. & al., (1937), Am. J. Anat. **61**, 56—61.
15. BILEWICZ, St., (1938), Z. vergl. Physiol. **26**, 90—96.
15a. BUSHNELL J., (1938), Anat. Rec. **72**, **97.**
16. CONRAD, D., (1917), Nos Bactraciens, Bruxelles.
17. DELCOURT R., (1938), Arch. Internat. Méd. Exper. *XII*, 499—515; *Id.*, (1939), ibid. *XIII*. 719—873.
18. DRAGOIN, J. & Crisan, C., (1939), Bull. Acad. Méd. Roum. **8**, 70—76.
19. GUDERNATSCH, J., (1912), Arch. F. Entwickl.-mech **35**, 28—49. *Id.*, (1914), Am. J. Anat. **15**, 28—49.
20. HAVAS, L., (1937a), Nature, Febr. **139**, 371.
21. Id., (1937b), Bull. Assoc. Fr. Cancer, **26**, I—28.
22. Id., (1938), Growth, *II*, 257—260.
23. Id., (1939b), Nature, **143**, 809.
24. Id., (1940b), Arch. Internat. Méd. Exper. *XV*, 1—44.
24a. HERZFELD, E. and Frieder, A., (1933), D. med. Wch. **49**, 84.
24b. LANCZOS, A., (1939), Arch. exp. Zellforch. **191**, 430.
25. KAWAMURA, T., (1939), J. Sc. Hiroshima Univ. **6**, 115—217.

26. KEPPEL, D. M. & Dawson, A. B., (1939), Biol. Bull. *LXXVI*, 153—164.
26a. LAUR C. M., (1938), Add. d'Anat. Path. **15,** 1—.
26b. Id., (1939), id. **16,** 196.
27. LEFEVRE, J., (1939), C. R .Acad. Sci. Paris, **208,** 301.
28. LEVAN, A., Hereditas, (1942), *XXVIII*, 244—245.
29. SÓS, J., (1939), Naunyn Schmiedeberg's Arch. **192,** 79.
30. SOYANO, J., (1940), Bot. Mag. Tokyo, **54,** 141.
31. WOLSKY, S., (1940), Magyar Biol. Kut. Munk., **12,** 1—3.
32. WOLSKY, S. & ALLODIATORIS, I., (1941), ibid. **13,** 456—558.

EFFECTS OF COLCHICINE INJECTED IN FEMALE DROSOPHILA.

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With 4 Figures and 1 Table in the text.

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In the following experiments I investigated the effect of different concentrations of injected colchicine on adult female Drosophilas and on their eggs when laid.

GELEI and CSIK (1940) established that when the Drosophila's food contains $1:10^3$, $1:10^5$, $1:2.10^5$ diluted colchicine, the flies die after a couple of days without offspring. The animals can survive the $1:3.10^5$ and $1:4.10^5$ dilutions, but with a smaller number of progeny than in the control cultures. HADORN (1946) found on dissecting the gonads of Drosophila larvae that one can treat them in vitro with colchicine and so transplant the gonads, the chemical agent causing a typical injury to them.

My present effort was to see if a similar effect could be produced in the young adult flies with injections of colchicine.

TECHNIC.

For the injection I employed Beadle—Ephrussi's standard method. I diluted the colchicine from a 1/1000th% basic fluid in sterile Ringer solution. I used the following 3 dilutions for the experiment: $1:10^5$, $1:2.10^5$ and $1:3.10^5$. For the injections I employed a 1 cm^3 "Tuberculin" syringe provided with a regulating screw-wheel. The wheel was divided into 36 degrees. A whole revolution of the wheel, i. e., 36°, ejected 0.01 cm^3 of the fluid. To fill the abdomen of a young female Drosophila an average turn of 2° was required. In any case the insects themselves regulated the amount of the fluid, because the membrane between the segments of the abdomen admitted no more liquid once it was full, so that if animals of one size are used the same amount of liquid can always be employed. Thus I can give approximate data on the amounts

of the solutions and the weight of the colchicine, namely, that one example can take approximately 0.00055 cm^3 fluid, and the weight of the colchicine so injected diluted to $1:10^5$ is 1/10th microgram; to $1:2.10^5$ is 1/20th microgram, and to $1:3.10^5$ is 1/30th microgram.

In each series 10—10 examples were injected at exactly the same time, one series when they were 1 hour old, one when 10 hours old, and one when 24 hours old. One series 1 hour of age was taken as control and injected with pure Ringer solution. I registered the production of eggs every 24 hours for 5—6 days.

From the deformed eggs I made histological slices, fixed in Carnoy, Delafield staining for 1 minute.

THE EXPERIMENTS.

The injection of the pure Ringer solution had no effect, either on the adult control examples or on their eggs. Under sterile treatment the perforation soon healed and the swelling of the abdomen disappeared in half a day, after which each example continued its normal existence.

The colchicine, according to the different concentrations, had different effects, and the differences in the ages of the examples had their influence too. The data thereon are given in the following Table.

a.) *Mortality.* Of the examples one hour old receiving colchicine in a concentration of $1:1.10^5$, 90% died. Of the 22 treated females only 2 remained alive at the end of 72 hours. Of the examples 1 hour old receiving the $1:2.10^5$ solution, not one died; of the 10 hour old series 3 examples died, of the 24 hour old, one.

b.) *Sterility.* The colchicine injected rendered the females either permanently or temporarily sterile. The permanent sterility was caused by the $1:1.10^5$ concentration. The 2 examples above mentioned which survived the strongest solution laid no eggs at all. Of the one-hour examples given the milder solution I observed that 2 females remained permanently sterile. In the case of those temporarily sterile I remarked that they did not begin to lay during the 48th hour of life but some 2—3 days later. Of the examples receiving the weaker solution when 1 hour old, among the 10 females 2 delayed laying by 3 days, 2 by 2 days, and 1 by 1 day. Under the same concentration the examples treated later in life were more resistant. Among the 10 females when 10 hours old, 3 delayed 1 day in laying, of those treated when 24 hours old, 8 delayed 1 day. Of those receiving the weakest solution ($1:3.10^5$)

conc.	age of fem. injected.	number of fem. treated.	mortalety	permanently sterile	temporarily sterile	deformed eggs in percent	daily production of eggs of 10 fem.
$1:1.10^5$	1^h	22	20	2	—	—	—
$1:2.10^5$	1^h	10	—	2	5	100.0	11
						20.0	20
						1.2	73
						3.4	88
						0.0	159
$1:2.10^5$	10^h	10	3	—	3	65.1	8
						10.6	43
						0.0	75
						0.0	131
						0.0	144
$1:2.10^5$	24^h	10	1	—	8	0.0	3
						0.0	146
						0.0	143
$1:3.10^5$	1^h	10	—	—	—	90.5	95
						15.2	327
						0.0	310
$1:3.10^5$	10^h	9	—	—	—	44.8	98
						20.2	232
						1.9	351
						0.0	260
$1:3.10^5$	24^h	10	—	—	—	0.0	88
						0.0	434
						0.0	428
Control.	1^h	10	—	—	—	0.0	112
						0.0	327
						0.0	265
						0.0	300

only 3 of the group treated when 10 hours old delayed 1 day in laying, all the others of the 2 groups began to lay their eggs at the normal time on the 2nd day.

In respect to mortality and sterility the general observation can be made that the animals show individual variations in their reactions to the colchicine. But, roughly speaking, it is clear that the mortality and sterility depend in direct ratio to the concentration of the colchicine. The number of animals investigated was not sufficient to establish to what extent the age of the animal influences the effect of the colchicine. Comparing the effect of colchicine as given by injections with treatment in vitro on the one hand and direct feeding of colchicine on the other, it can be seen that it stands somewhere between the two.

c.) *Number of eggs laid.* The average daily egg production of 1 female is given, as evaluated from the average daily production of 10. The number of eggs laid the first 3 days was indicative of the intensity of egg-production. The $1:2.10^5$ concentration gave a small number but increasing slightly according to the advance in age. Under the effect of the $1:3.10^5$ concentration the behaviour was scarcely different from that of the normal controls (Fig. 1).

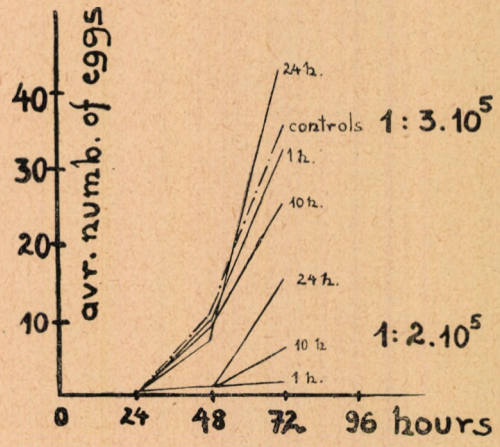

Fig. 1. The average daily egg-production of a treated female in the groups of $1:2.10^5$ and $1:3.10^5$. The average production of the control is represented by the line -.-.-.-.

d.) *Deformed eggs.* The most typical effect of the colchicine was seen in the eggs. Depending on the concentration and age, a definitely determined percentage of misshapen eggs appeared, while not a single deformed egg was present among those laid by the controls (1004 eggs). The shape of the deformed eggs was typical of each given concentration.

The consequence of the $1:2.10^5$ solution was that the 2 long spongy horns typical of the normal egg disappeared entirely and the shape of

the egg lengthened (62 cases). After the 1:3.10^5 solution the long horns also disappeared but a small protusion remained on the dorsal side of the egg (Fig. 2.). (241 cases.)

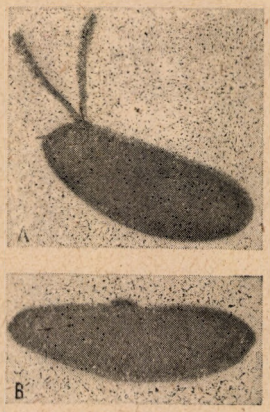

Fig. 2. The normal (A) and the deformed (B) eggs after treating with colchicine (1:3.10^5).

I kept the deformed eggs at a temperature of 25° C to see whether they were capable of producing larvae or not. This did not occur, in a single case.

From the histological examination it appears that the tumour-like protrusion results from an accumulation of chorion cells. In substance and structure it is the same as in the horn of the normal egg, though it is only one, not a pair, and considerably less in number of cells. These empty chorion cells are larger at the base of the protrusion, while those at the top are smaller. The interior of the egg suffered injury in that the differentiation of the tissues stopped.

The percentage of deformed eggs in relation to colchicine concentration and age can be seen in the two following *graphs,* both of them typical in that the older animals better resist the treatment, and with stronger concentration the injury is greater. Further characteristics are that the quite young, 1 hour old, animals react very actively to the colchicine, but the effect quickly wears off. The examples treated when 10 hours old reacted less actively but the effect wore off more slowly.

It appears from these investigations that with the injection of colchicine into the Drosophila organism a functional disturbance is caused which can be said to resemble the effect of colchicine when administered in their standard food. The effect of colchicine when

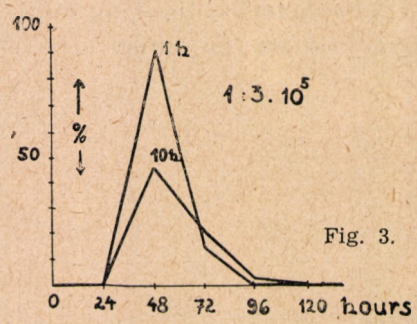

Fig. 3.

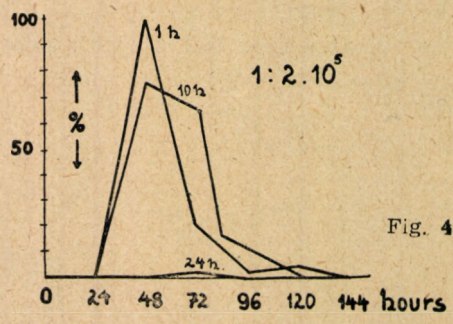

Fig. 4.

Fig. 3. The percentage-axis of deformed eggs after treating the 1 hour and 10 hour old animals with colchicine ($1:3.10^5$).

Fig. 4. The same as Fig. 3. but in the case of a stronger ($1:2.10^5$) colchicine solution.

injected produces 2 factors which cause a reduction in progeny: 1.) the mothers become completely or temporarily sterile; 2.) deformed, and so unproductive, eggs are present to a certain degree. But while the effect of injected colchicine wears off after a few days, the colchicine administered to the Drosophila in food, if not in lethal concentration, causes a permanent though lower equilibrium in the number of progeny, which has been established by GELEI and CSIK in respect to certain degrees of concentration. It is probable that there do not exist in the alimentary system of the Drosophila the sort of enzymes which can destroy the colchicine.

On the other hand, comparing our investigations with those of HADORN, it appears that the ovarium of the newly born females until they reach the age of 24 hours is as sensitive as that of the larva. The characteristic and typical deformation of the eggs indicates the same effect of the colchicine as in the larval stage and in the early adult life.

SUMMARY.

Injections of $1:1.10^5$, $1:2.10^5$ and $1:3.10^5$ concentrations of colchicine in female Drosophila imagos aged 1 hour, 10 hours and 24 hours caused injury in respect to viability and fertility. Moreover, typical injury-effects were to be seen in the eggs when laid, depending on the age when treated and the degree of concentration of the colchicine.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for the English translation.

REFERENCES.

GELEI, G. and Csik, L. (1940). *Biol. Zbl.* **60.** 275.
HADORN, E. (1946). *Rev. Suisse de Zool.* 53. 486.

A NEW METHOD FOR NARROWING MICRO-CAPILLARIES.

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With 1 Figure in the Text.

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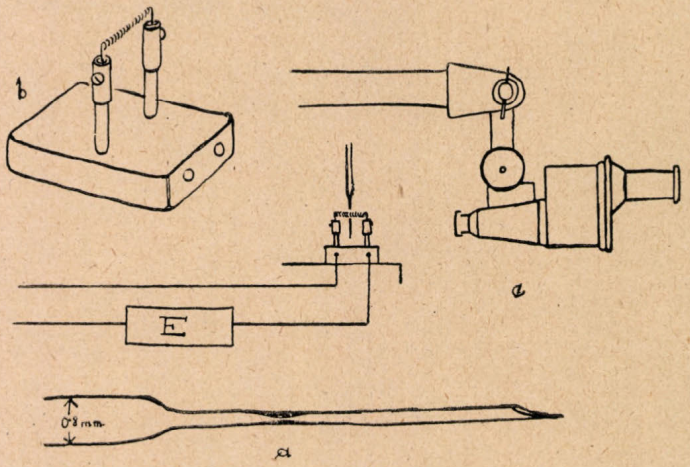
Narrowing of glass capillaries can be accomplished, according to previous descriptions, with horizontally opposite burning microflame. The apparatus described hereafter brings about this narrowing with the help of an electrically heated spiral instead of a microflame. The advantage of this method is that the degree of narrowing can constantly be controlled under binocular microscope, and further, very precise bends and curves can be given to the micropipette, a thing which is often necessary.

The following drawing shows the construction of the apparatus.



The heated body is a wire spiral 15 mm long and 1.5 mm in diameter (b). The usual 0.1 mm heating filament can be twisted to make this spiral. The heat can be supplied by the ordinary current if the right resistance is employed. The white-hot spiral is installed horizontally

in the field of a binocular microscope. (c). The drawn-out capillary to be narrowed approaches from the side in a downward, perpendicular direction, with the help of a PÉTERFI-JANSE micromanipulator. The narrowing of the capillary walls (a) can be observed through the microscope melting under the heat and when we reach the desired constriction it can be removed at the required moment from the vicinity of the heated body. Curves can be made in the capillary if it approaches the heated body horizontally from above, until the curve reaches the degree desired.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for the English translation.

MEASUREMENT OF THE PHOTODYNAMIC EFFECT OF CANCEROGENIC SUBSTANCES ON BIOLOGICAL INDICATORS (DROSOPHILA).

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With 1 Table in the text.

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According to the observations of Tappeiner, Jodlbauer, Pincussen, Sacharof, Sáchs, Neuberg (1925) et al., micro-organisms, cells or tissues treated with fluorescent materials and irradiated with a definite wave length perish. The active substances in this process have been called sensibilizing agents and their effect photodynamic effect.

It is known that the three most widely used cancerogenic substances, 3—4 benzpyrene, methylcholanthrene and 1, 2, 5, 6 dibenzanthrancene, show a yellowish-greenish-blue fluorescence in ultra violet light. If these substances get into the cells they act after u. v. irradiation as photosensibilizing agents. The photodynamic effect of different cancerogenic substances has already been the object of several experiments.

Doniach (1939), working on the photodynamic effect of 3—4 benzpyrene, 1—2 benzanthracene, cholanthrene, methylcholanthrene on paramaecium, found that these substances have a stronger effect than non-cancerogenic photosensibilators. Graffi (1940), working on Ciliata and Chorethra larvae, found that if they absorb cancerogenic substances they burst or die after u. v. irradiation. Daphnia support radiation for a longer time than the Ciliata and the Chorethra.

Hollaender, Alexander, Peter A. Cole and F. S. Brakett (1939) observed the toxic effect of methylcholanthrene after u. v. irradiation on yeast cells. Biscegli and Grazía (1936) described the slowing up of growth after illuminating tissue cultures treated with benzpyrene. Lewis (1935) found similar results as the effect of methylcholanthrene and dibenzanthracene on fibroblast cultures of the heart.

Wollman (1940), Mottram and Donaich (1940) mixed the three mentioned substances with blood in vitro and irradiated it with sun

light or u. v. light. As a result a great haemolysis of the blood cells occurred. According to WOLLMAN, haemolysis occurred the quickest as a result of benzpyrene treatment. Methylcholanthrene had a slighter, and dibenzanthracene the slightest effect.

GRAFFI (1944) observed heavy toxic skin lesions in rats after a single benzpyrene painting and exposure to sunlight. The effects of this skin lesion were the epidermis and acute inflammation of the subcutis.

MOTTRAM and DONIACH (1937) painted the skin of mice with benzpyrene solution and exposed the animals to sunlight. The skin reddened in the area of the painting, there was edema, and the next day dermatitis. Under the irradiation the animals avoided the sunlight and was nervous.

MORTON, LUCE-CLAUSEN and MAHONEY (1940) observed that in animals treated with benzpyrene and kept in the dark the swelling appeared earlier than in animals kept in the light. MOTTRAM and DONIACH (1940) thus explained the phenomenon: In consequence of the toxic effect of the cancerogenic substance the already injured cells perish faster under photodynamic influence, and give no time for the cancerogenic action to come into play.

From the above experiments it can be seen what photodynamic effect the different cancerogenic substances have on different microorganism, cells, tissues and organs if exposed to u. v. or sun light. But the photodynamic effect has not yet been accorded a numerical valuation.

In our present experiments we tried to express this photodynamic effect of cancerogenic substances in numerical values according to standard biological procedures.

THE EXPERIMENTS.

For our experiments we used light-protected methylcholanthrene, 3—4 benzpyrene and 1, 2, 5, 6 dibenzanthracene. We carried out the experiments on a stock of 5 year pure inbred white-eyed Drosophila (white I. chr. 1.5 MORGAN unit). The animals were kept in the dark throughout the entire experiment. The controls laid their eggs on a nutriment of rich yeast mixed with standard Drosophila food. The treated larvae were obtained by mixing 0.1 g. cancerogenic substance with 13 g. standard food, on which the imagos laid their eggs. We used the III stadium larvae just before the pupa stage, without regard to sex.

For the irradiation a quartz lamp (Hanau 2.5 A, 220 V.) was used, at a distance of 36 cm. The lamp was cooled with a ventilator. The temperature was kept between 24 and 26°. The larvae were irradiated

in a Petri cup in the bottom of which was wet white filter paper. We measured the time which elapsed from the beginning of the irradiation until the animal's death.

We made the experiment on 4 groups: the first, of animals treated with benzpyrene, the second with methylcholanthrene, the third with dibenzanthrancene, the fourth being the controls. From each group of larvae 5—10 were examined through fluorescence microscope. We found that in the food in the intestines of the larvae the crystals of cancerogenic substance until they dissolved were visible in the ventral cavity in their original luminescent colours. The substances dissolved and absorbed in the tissues and cells showed a luminescence of a different colour. (We accounted for this partially in another communication.) We further established that the same organs in the larvae always absorbed the different cancerogenic substances, and in these organs the luminescence was equally diffused.

Equally, in every group, as the u. v. light began to affect them, the larvae made lively movements in an effort to escape, later moved more slowly, then bent and made small snake-like movements, until all movement ceased. To avoid all possibility of error the larvae were observed for a long time after the radiations to make sure movement had really ceased. The next day they were re-examined to make sure they had not pupated. We did not observe such cases.

The time taken for our animals to die was calculated in averages, which we give in the following Table:

TABLE I.

Number of Animals	Composition of Food	Average time required to die
40	13 g. standard Drosophila food + 0.1 g. benzpyrene	6 minutes, 30 seconds ± 1 minutes, 15 seconds
40	13 g. standard Drosophila food + 0.1 g. methylcholanthrene	13 minutes, 35 seconds ± 1 minutes, 58 seconds
39	13 g. standard Drosophila food + 0.1 g. dibenzanthracene	19 minutes, 34 seconds ± 3 minutes, 33 seconds
40	13 g. standard Drosophila food	39 minutes, 51 seconds ± 5 minutes, 12 seconds

DISCUSSION.

We must first consider whether Drosophila larvae are suitable as biological indicators for the present experiment, meeting generally accepted conditions. Holthusen and Braun (1933) on the basis of bio-

logical studies in radiation have recapitulated the general conditions necessary for biological test objects. They should be of small size, homogeneous in large numbers, varying but slightly, accessible at all times in masses of individuals, and capable of showing sharp reactions. Age and temperature also play important roles. In Drosophila eggs, according to PACKARD, experimental error lies within 3%. For our requirements too the Drosophila is the generally accepted, well known, extraordinarily well elaborated object, with highly developed breeding technics, fulfilling most of the above conditions — size, variablity, etc. Variations in temperature and ventilation of 2—3 degrees make no significant difference. The rich and uniform distribution of the crystals in the food was destined to assure an evenly diffuse distribution in the organs of the larvae. The slight variation of the ± value of the experimental results shows that the exterior modifying factors were minimum. So our material meets the requirements demanded of a biological indicator and gives us the means of considering the data from other standpoints as well.

It is apparent from our experimental data that benzpyrene has the strongest effect on Drosophila larvae. Then follows methylcholanthrene and then the dibenzanthracene. If we make a small allowance we can say that the methylcholanthrene has half, dibenzanthracene one-third the photodynamic effect of benzpyrene. That is, the animals treated with methylcholanthrene must be irradiated twice as long as those treated with benzpyrene, those treated with dibenzanthracene three times as long, for death to ensue.

Death occurs in the animals treated with benzpyrene in 1/6th of the time it takes for the control animals to succumb to u. v. rays. That is, only 1/6th of the u. v. irradiation necessary to destroy untreated animals suffices for those treated with benzpyrene. The methylcholanthrene-treated succumb in 1/3d the time required by the controls, in the dibenzanthracene-treated in 1/2 the time.

If we compare in order of strength the photodynamic effect expressed in Drosophila units with WOLLMAN's experiments, we see that he established similar order in red blood cell haemolysis, as benzpyrene haemolyses red blood cells in the shortest time for one unit of quantity, followed by methylcholanthrene, last of all by dibenzanthrancene. From his and our experiments we can conclude that the speed of the process of photodynamic effect is the same chronologically in the cells and in the whole organism, acting characteristically on the different substances.

How this effect takes place, by what mechanism, our experiments

do not disclose. On the basis of other studies only two explanations are probable. BOYLAND (1933) in his work produced from cancerogenic substances irradiated with u. v. rays alkali-soluble oxidised materials which strongly hindered the respiration of the tissues, specifically injured the enzyme functions. It is possible that a similar process takes place in the Drosophila larva and leads to death. But it is also possible that the direct cause of death is not the photoproduction of the cancerogenic substances but the process of coagulation stimulated by the u. v. rays in the tissue protein and repeatedly speeded up by the cancerogenic substances as photocatalisator.

It seems worthy of mention that if we compare the time required for the cancerogenic substances in question to induce a swelling with the strength of the photodynamic effect observed in our experiments, we see that in both cases the dibenzanthracene has the weakest effect, while the two stronger cancer-causing substances show a stronger photodynamic effect here too. According to the statistics of FIESER (1938), methylcholanthrene has the strongest cancerogenic effect, with benzpyrene slightly weaker. In our studies the benzpyrene had the strongest effect, the methylcholanthrene proving to be only half as strong. We should point out that FIESER's statistics were not based on animals kept from the light.

Our experiments made possible the biological standardization of photodynamic effect on Drosophila larvae and these biological standards we can now range beside the different cancerogenic substances as characteristic values and assemble them in order of their strength. Thus the photodynamic strength of the chemical substances suitable to our experimental procedures can be expressed numerically. Naturally, a recognition of these procedures means that they are suitable not only for measuring the photodynamic effect of cancerogenic substances alone, but of other substances as well. It should be noted that only those substances are suitable which in the dark have no other pathological effect on the larvae.

SUMMARY.

1.) Cancerogenic substances were introduced in the organisms of Drosophila larvae through their food, and their photodynamic effect measured under u. v. irradiation.

2.) Benzpyrene had the strongest effect, after which came methylcholanthrene, and last dibenzanthracene.

3. On the basis of this experiment it is possible to measure and standardize the photodynamic effect of chemical substances on Drosophila larvae in the III stadium.

The three cancerogenic substances were presented by the Hoffmann la Roche Co., Basel. The authors have pleasure in expressing their thanks for this gift.

Grateful acknowledgment is made to Mrs. J. Thompson Vass for the English translation.

REFERENCES.

Biscegli & Grazia (1936). *Act. Canc.* **2.** 355.
Boyland (1933). *Bioch. Journ.* **27.** 791.
Doniach, I. (1939). *Brit. J. Path.* **20.** 227—255.
Doniach & Mottram (1940). *Nature.* 748.
„ „ (1940). *Amer. J. Canc.* **39.** 234—240.
Fieser (1938). *Amer. J. Canc.* **34.** 72.
Graffi, A. (1940). *Zscht. f. Krebst.* 50. H.
„ (1944). Magy. Orv. Arch. XLV. 3.
Hollaender, A., Cole, P. A. & Brakett F. S. (1939). Amer. J. Canc. 265—272.
Holthusen, H. & Traun R. A. (1933). *Grundlagen und Praxis der Röntgenstrahlen-Dosierung.*
Lewis, M. R. (1935). *Amer. J. Canc.* **25.** 305.
Morton, J. J. Luce-Clausen, E. M. & Mahoney, E. B. (1940). *Amer. J. Roentgenol.* **43.** 896—898.
Packard, C. Radiology, 27. 191
„ „ **29.** 12
Pincussen, (1925). *Handbuch der Biochemie.* S. 739. Bd. II.
Wollmann, M. (1940). *Nature.* I. 592.

THE EFFECT OF 3—4 BENZPYRENE IN RESPECT TO THE NON-DISJUNCTION FREQUENCY IN DROSOPHILA MELANOGASTER.

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With 4 Figures and 1 Table in the text.

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In considering the well-known theory that cancer originates from mutation, it is important to know what special effects cancerogenic substances may have on mutation. On the Drosophila, the best known genetical experimental material so far tried, cancer-causing hydrocarbons have been used to good purpose in this respect. *Auerbach* and *Friedrich-Freksa* (1940) have dealt with such Drosophila experiments in certain of their publications. They did not find that cancerogenic substances changed the gene mutation in Drosophila, which they explained as due either to the local concentration being scanty, or to the time during which the substance could act being insufficient.

In the following pages we deal with experiments in which we examined the dispersal of a cancerogenic hydrocarbon, benzpyrene, in the Drosophila organism under different life cycles, and investigated how its presence in the Drosophila egg may have affected the non-disjunction of the X-chromosomes.

TECHNIC.

Our investigations were carried out on 5-year, pure inbred, white-eyed stock, the animals constantly protected from rays of light during the experiment, at a temperature of 25°. We found the best means of introducing the substance was to mix it, in the form of minute crystals, in the standard Drosophila food. To the food of the imagos 30 mgr. benzpyrene was mixed with 4.5 gr. thin, yeasty food. This mixture

was smeared on pieces of filter paper the size of a postage stamp. By this method of feeding, benzpyrene crystals were constantly in the intestinal canals of the larvae or the imagos, and so there was constant absorption of benzpyrene into the tissues.

The presence of benzpyrene in the tissues and organs was established by flourescence microscope. The absorbed benzpyrene showed a pale blue fluorescence in the cells and tissues. Whole larvae and dissected organs in Ringer solution were also examined.

BENZPYRENE IN THE DROSOPHILA ORGANISM DURING THE DIFFERENT LIFE CYCLES.

We used a strain of specimens taken from white stock for the experiment, raised on a constant benzpyrene diet, in the dark. The Drosophila, raised in the dark on the constant benzpyrene diet showed no toxic effects. We followed this stock through 6 generations — each generation receiving the constant benzpyrene diet — and examined about 2000 individuals without finding any sort of abnormality which would have indicated a disturbance in the development.

It could be established in one cell, in relation to benzpyrene absorption, that the vacuoles and granules of the plasma showed lively, strong fluorescence, the nucleus none whatever. In the Drosophila the large, flat, 6-sided fat -cells and the malpighi tube cells are particularly suitable for such observations. (Fig. 1.) Our observations here coincide with those of GRAFFI (1939.), who, with another experimental object in view, also got benzpyrene dispersed in the plasma but not in the nucleus. It is possible that some special chemical substance in the cell nucleus "defends" it from the benzpyrene fluorescence, or that the benzpyrene molecules do not permeate the membrane of the nucleus; or again that such a small amount of benzpyrene is present that the fluorescence cannot be seen. In general our studies of the Drosophila so far show that the benzpyrene accumulates in the fat-containing granules and vacuoles of the cells, in the plasma dispersing uniformly, and its appearance in the organs also conforms to this fat-attracting phenomenon.

In the intestinal canals of larvae of the I or II stadium deposited on benzpyrene medium, solid benzpyrene crystals could be seen from the first day. 24 hours later a blue fluorescence diffused the whole organism, which shows that the absorption of benzpyrene began in the organs of the larvae. The systems of the control larvae, without benz-

pyrene, also showed a slight fluorescence in the ultra violet light of the fluorescence microscope, but this fluorescence was very light and greenish, in contrast to the benzpyrene-treated specimens, which were alight with a strong blue colour. We undertook partial examinations of the III. stadium larvae before pupating. (Fig. 2.) Decidedly strong fluorescence could be seen in their fat-bodies. Equally strongly fluorescent were the walls of the intestinal canal and malpighi tubes. Less strong, yet well differentiated from the controls, was the benzpyrene fluorescence in the imaginal buds and the cerebral ganglions. No trace of benzpyrene could be established in the salivary glands or in the sexual organs of either sex. While, for example, the fat-lobes adhering to the salivary glands were lighted by a bright blue fluorescence, the tissues and cells of the salivary glands themselves showed absolutely no fluorescence. In exactly the same way, the fat lobes surrounding the sexual organs were remarkably fluorescent, while the sexual organs themselves were entirely unilluminated.

The fluorescence continued after the larvae treated with benzpyrene had pupated. During the entire pupal life, with its complete metamorphosis, the blue fluorescence did not disappear. The accumulation of benzpyrene could be so marked that its blue light shone through the chitinous surface of the purparium under ultra violet irradiation, while only a greenish light could be seen in the untreated pupae. Just before emerging too, dissection of the pupae showed that it was primarily the fat cells and the intestinal canal that were lighted up.

The newly hatched imagos carried the benzpyrene on from the larval and pupal life and kept it for a time. If the imagos which had been fed on benzpyrene in their larval life were changed to a normal diet on hatching out (as imagos) the benzpyrene could still nevertheless be seen in their organisms for 72 hours. The benzpyrene could be seen in the fat cells, the intestinal canals and malpighi tubes of females 1—2 hours old, and in the developing oöcytes in females 12 hours old. After 24 hours the fluorescence in the fully developed ovaries could scarcely be distinguished from that of the controls, but the malpighi tubes and intestinal canals were still fluorescent. After 72 hours only the malpighi tubes showed traces of the benzpyrene.

Even if the larvae had not been fed on benzpyrene the imagos' organisms absorbed it to a high degree if they were given a mixture of benzpyrene and yeast. Here too the organs above mentioned took up the benzpyrene; only in the muscular system no trace of fluores-

cence could be seen. The most remarkable is the strong, bright fluorescence of the ovaries of the females specimens under this treatment. (Fig. 3.) The male gonads do not take up as much. The eggs deposited by females fed each day on fresh benzpyrene medium were always alight with a bright blue fluorescence, (Fig. 4.) while the control eggs gave a greenish light under the ultra violet rays. In the older eggs (about 12 hours) where the developed embryonic tissues also began to differentiate, the embryonic tissues took a dark blue fluorescence calour. The penetration of the benzpyrene into the eggs takes place in the ovarioles.

The first stage larvae emerge from the fluorescent egg completely viable and carry the benzpyrene fluorescence on with them, without further treatment. This lasts for 6—12 hours. After that time the benzpyrene disappears unless the treatment is repeated. But if they again get on to a benzpyrene culture medium the cycle begins again, as described above.

In summarizing the foregoing we can assert that:

1. Benzpyrene can be taken up by the Drosophila in any stage of the animal's development with the food.

2. During metamorphosis the substance is carried over from one phase to the other.

3. The benzpyrene taken by the mother arrives through the eggs in the I. stadium F/1 generation larvae.

GENETICAL INVESTIGATION.

As we mentioned in our introduction, the aim of our genetical experiments was to inquire into the significance of the presence of benzpyrene in spontaneous non-disjunction frequency. In the present communication we need not enlarge on the phenomenon of non-disjunction itself, so well known from the publications of MORGAN and many others, only touching on the details which seem necessary.

In the organisms of the specimens used in our experiments the quantity of cancerogenic substance taken was apportioned so that it must still give fluorescent light in the oöcytes of the newly hatched females which had been given benzpyrene in the larval state, this fluorescence being due to 13 gr.: 10 cgr. in the food. When it was the imagos which were treated, we wanted the laid eggs to have the same fluorescent colour. That this did take place we were convinced by tests carried out every 2 or 3 days under fluorescence microscope. (4—5 gr. food: 30 mg. benzpyrene).

For crossing we used w/w genotype females and B/y genotype males (Progeny to be expected: red-Bar phenotype females, white-normal males; the exceptions: white normal females, red-Bar males.).

We carried out 2 experiments. In the first (Table I.) we treated the females only in the larval state, immediately after hatching transferring them to a culture without benzpyrene. The males were not treated at all. Each female laid eggs for 8 days. There were 12 cultures with 2—2 females. In the other experiment (Table II.) both the males and females were kept on a benzpyrene culture medium, renewed every 24 hours. Here each female laid eggs during 2 to 4 weeks. We kept the control flies, receiving no benzpyrene, under similar conditions throughout the experiment. (Table III.)

The results of the experiment are shown in the following Table:

Treatment	Number of treated females	Offspring				Total ex. %
		Reg.	Ex.	Reg.	Ex.	
in larval life (I)	24	1190	2	1096	7	0·39±0·13
in adult life (II)	10	1235	—	998	—	
	15	1290	2	1167	—	
	25	2525	2	2165	—	0·04±0·03
Controls (III)	24	1075	2	1049	3	
	10	1466	5	1188	1	
	15	1470	1	1200	3	
	49	4011	8	3427	7	0·2±0·05

According to the above Table, in the control stock the exceptional individuals are present in the frequency of 0.2±0,5%, i. e., the proportion between the exceptions and the regular types is about 1:500. That this high percentage is not a chance effect can be assured by the fact that the total (7463) control progeny were created in three successive breeds and showed equally high percentages in all three steps. (1st control: 2124 regular, 5 exceptions, 2nd control: 2654 regular, 6 exceptions; 3d control: 2670 regular, 4 exceptions.) The 0.2±0,5% can thus be regarded as standard, by which to compare the results in the treated cultures.

In the first experiment, where the females were treated in the larval stage, there is no significant difference between the control and the treated stocks as to proportion of exceptional animals. (0,39±0,13.)

Under the second treatment, among the progeny from the 4692 eggs

of benzpyrene-treated parents, there was not a single exceptional male and we found 2 exceptional females brought about by the non-dividing of the X-chromosome. The proportion here is 1:2300. Expressed in percentage, the progeny hatched from the benzpyrene eggs was $0.04 \pm 0.03\%$ of exceptions. The difference between the control and the 2nd experiment was $0.16 \pm 0.05\%$ in respect to presence of exceptional animals. This difference (0.16) is 2.7 times greater than their standard error (0.059). In any case such a value makes it extremely likely that there is really a difference between the control and the 2nd experiment.

DISCUSSION.

The non-disjunction of the X chromosomes usually occurs with uniform frequency in groups taken from the same stocks in the manner of the gene mutation. This spontaneous proportion depends primarily on the genes of the stock, but after all in certain cases the physical and chemical state reigning in the plasma gives the last impulse when the reduction division of the cell takes place. This has been proved by well-known experiments in which heat, X-rays, as well as colchicine, (Gelei-Csik, 1940) have brought about changes in frequency of non-disjunction. From these studies we might expect that in our investigations of a chemical substance, benzpyrene, in the plasma the number of non-disjunctions might also be affected. In judging the results of our experiments from that standpoint, however, we must keep in mind the difference between the conditions of the lst and 2nd experiments; for in the lst experiment fluorescence microscopic observation disclosed that the mothers treated in the larval state had benzpyrene only in the young oöcytes. The eggs when laid were not fluorescent. Thus this type of pre-treatment could not be effective, nor do we find a significant difference from the standard values.

But in the 2nd experiment benzpyrene was constantly present, not only in the eggs developing in the ovarioles, but also in the eggs, when laid. Thus the benzpyrene was also there in the plasma during the egg's reduction division. This might lead us to expect that the chemical substance would have some effect on the standard number of non-disjunctions. And in reality it turned out that we got fewer exceptional progeny from the benzpyrene-treated eggs than were to be expected even of the control specimens. The statistical valuation showed the difference very strikingly; and further, if we consider the apparent proportion of exceptions in the 3 control cultures and the lst experiment

(1:500), and the exceptions among the progeny hatched from benzpyrene-treated eggs (1:2300), then we must conclude that the presence of benzpyrene actually diminishes the X-chromosome's non-disjunction frequency.

In a negative direction it is more difficult to determine changes in the usual proportions of spontaneous mutation, as we know of few similar cases which could be used for comparison. BERG and SEVERTZOFF (1941) observed in intraspecific hybridization the degree of descent in spontaneous mutation: IGNATIEV and SHAPIRO (1943) experienced the same on the effect of modifying factors; and according to ZAMENHOF (1943): "a decrease in the mutation rate of an unstable Drosophila gene has been effected by adding copper sulphate, sodium hydroxide, or ammonium hydroxyde to the food." We consider it imaginable that the stocks we used in our investigations may also be standard for non-disjunction, which after all depends on the genotype of the stock, the benzpyrene diminishing the gene mutation in the way ZAMENHOF describes in the case of other chemicals.

Another possible explanation is that the benzpyrene so affected the plasmatic condition of the eggs by way of physico-chemial, e. g. viscosity changes, that it "helped" the separation of the X-chromosome's from the synapsis.

Our experiments warrant the conclusion that benzpyrene, a photo-active cancerogenic substance, causes a change in the mechanism of heredity.

Whether this change, the decrease in the frequency of the non-disjunction, has an influence on the development of cancer or not should be investigated with further experiments.

Acknowledgements:

The photographs were made by Dr. M. GERENDÁS and we take this occasion for acknowledging our gratitude.

For the benzpyrene we are indebted to the HOFFMAN la ROCHE Co. Ltd. Basel.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for the English translation.

SUMMARY.

1.) A cancerogenic substance, benzpyrene, can be taken up by the Drosophila in any stage of the animal's development with its food, and its presence demonstrated by fluorescence microscope.

**

2.) During metamorphosis the substance is carried over from one phase to the other.

3.) The cancerogenic substance is absorbed from the body of the mother through the ovarium into the eggs, and so can be seen in the eggs when laid and in the Ist stadium larvae.

4.) The presence of the cancerogenic substance in the egg diminishes the X-chromosome's non-disjunction frequency.

REFERENCES.

AUERBACH Ch. (1940). *Proc. Roy. Soc. Edin.* **60.** 164—173.

BERG R. L. & Sewertzoff, A. N. (1941). *C. R. (Doklady) Acad Sci. URSS.* **32/3** 21"—215.

FRIEDRICH-FREKSA H. (1940). *Biol. Zbl.* **60.** 498—524.

GELEI G. and Csik, L. (1940). *Biol. Zbl.* **60.** 275—286.

GRAFFI A. Zschr. f. Krebsforsch. (1939). Bd. 49.

IGNATIEV M. V. and Shapiro, N. T. (1945). *C. R. (Doklady) Acad. sci. URSS.* **46.** 122—26.

MORGAN F. H., Bridges, C. B. & Sturtevant, A. H. (1925). *Bibliogr. Gen. II.*

ZAMENHOF S. (1943). *Rec. Genet. Soc. America* No. **12.** 57—58.

EXPLANATION OF THE FIGURES.

Figure 1. The intestinal canal of an adult female Drosophila treated constantly with benzpyrene. "A" intestinal canal with brilliant benzpyrene crystals. "B" fat cell. "C" malpighian tube. Photograph under ultra violet light.

Figure 2. Larvae of Drosophila treated with benzpyrene under ultra violet light. The control is slightly illuminated by its neighbour.

Figure 3. The ovaries of an adult female Drosophila treated constantly with benzpyrene (the two luminous parts left and right), the dark ones are the two controls. Photograph under ultra violet light.

Figure 4. Eggs of Drosophila maintained on food containing benzpyrene, under ultra violet light. In the middle are three dark controls.

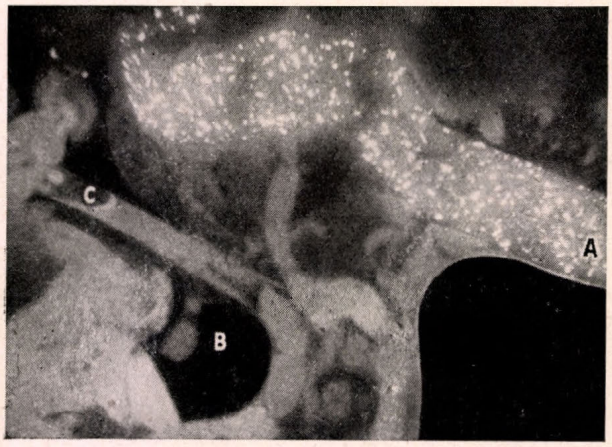

Fig. 1.

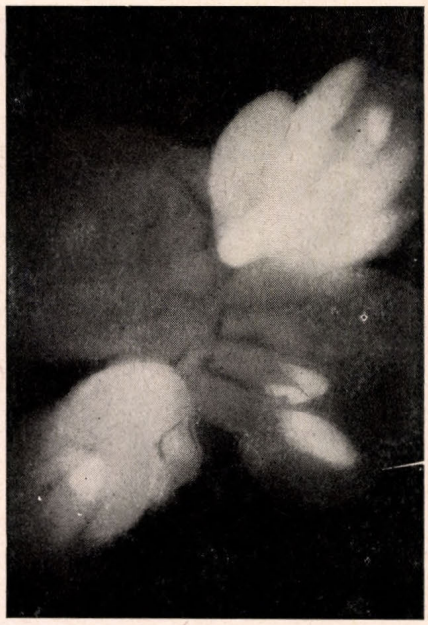

Fig. 3.

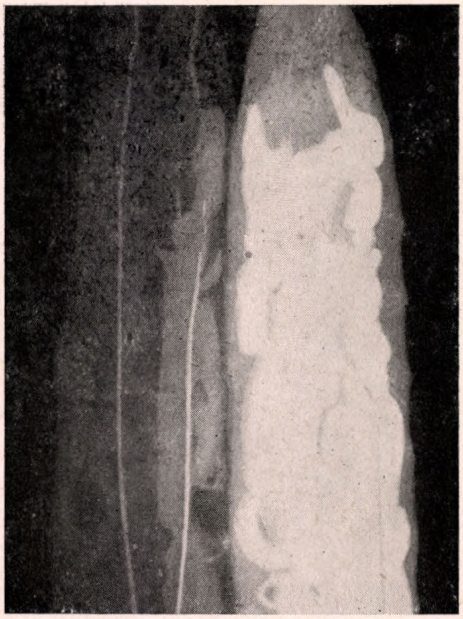

Fig. 2.

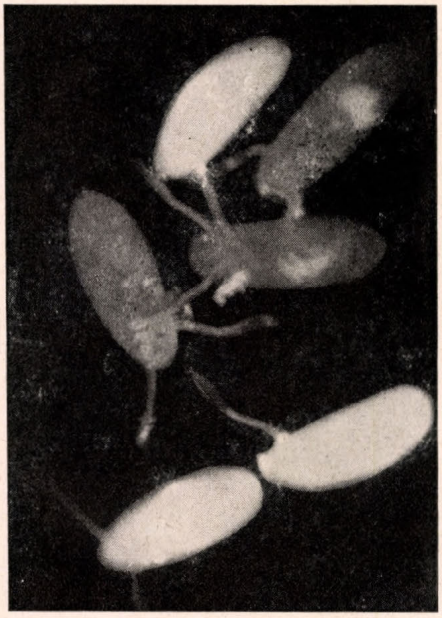

Fig. 4.

INVESTIGATION OF THE EFFECT OF 3—4 BENZPYRENE ON AMPHIBIA.

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(With 2 Figures in the text.)

(Received for publication 1st June 1944.)

The effect of cancerogenic substances was first investigated on mammals, and it was found that they had a specific, cancerogenic effect on certain tissues and organs. Later the specific effect was determined on various species; that is, some had an inclination, others were resistant, to cancerogenic materials (Dittmar, 1939).

Amphibia were examined in respect to cancerogenic effects by Koch, G. B. Schreiber, G. Schreiber and F. Duran-Reynals (1939). Koch and Schreiber & Schreiber noted a development of neoplasm in salamanders when 3—4 benzpyrene dissolved in oil was injected subcutaneously. Neoplasm also appeared in the lungs and heart. On the other hand, in Duran-Reynal's experiments tumour was not observed when cancerogenic material was injected intramuscularly. Only necrosis of the tissues could be seen.

Because of these contradictory results we again investigated whether tumour could be produced in amphibia, or whether they were resistant.

In our experiments 3—4 benzpyrene was applied in various solutions, injected or painted on different organs. We examined not only the cancerogenic effect but also the changes in the tissues caused by histological methods. The presence of 3—4 benzpyrene in the organs was followed macroscopically under ultra violet light and in sections by fluorescence microscope.

MATERIAL AND TECHNIC.

Rana esculenta and *Triton cristatus* were used for the experiments. The following benzpyrene solutions were employed:

1.) o. 3 per cent solution of benzpyrene in acetone.

2.) o. 5 per cent solution of benzpyrene in olive oil.

3.) Colloid solution: 0.01 gr. benzpyrene dissolved, boiling, in 1 ml. glycerine, 1 drop in 1 ml. normal horse-serum, (30 drops = 1 ml.), shaken for half an hour. (GRAFFI A., 1939.)

The control and the treated animals were kept separately in glass vessels at room temperature. The height of the water was 15—20 cm. Water was changed every 3—4th day. The animals were kept from sunlight. The frogs were given no food, the salamanders were fed with meat.

The organs of each animal were examined macroscopically for pathological alterations. An ultra violet lamp was used in searching for the benzpyrene fluorescence. Heart, kidneys, liver, lungs underwent histological analysis, in some cases also the spleen, stomach, intestine, muscle and skin. The organs were fixed in 4 per cent solution of formaldehyde and were put through alcohol for paraffin embedding. The sections cut at 10 μ were stained with haematoxylineosin, Van Gieson's and Mallory's methods. One of the kidneys, a piece of the liver and of the skin were sectioned frozen, and examined, partly fluorescence microscopically, partly Sudan stained.

THE EXPERIMENTS ON THE SALAMANDER.

Treatment by painting. 7 salamanders were painted 3 times weekly with a 0.3 per cent solution of benzpyrene in acetone on the skin of the back for 4 months. About 3 to 4 drops of the solution were applied. 3 controls were painted at the same time with pure acetone.

During the experiment the skin peeled off repeatedly. The painted area had a yellowish fluorescence in u. v. light. At the end of the experiment the animal's skin showed no tumour development under histological analysis. Only necrosis could be observed, and in some places regeneration of the basal layer.

Treatment with colloid solution. 17 salamanders were injected with 0.5 ml colloidal benzpyrene solution under the abdominal skin 3 times weekly. 3 were injected with a similar solution but without benzpyrene.

In 7 salamanders, decapitated on the 30th day, pathological examination showed that the kidneys were anaemic and the liver very flabby, with a greenish-brown colour. The lungs were opalescent and anaemic. Macroscopically in u. v. light the kidneys were seen to be pitted with numerous small greenish-yellow points. Under fluorescence microscopic examination a deposit of benzpyrene was established in the cortex of the kidneys and in the endothelial cells of the renal tubules.

Under light microscope it was to be seen that the structure of the liver dissociated. The organs of the control animals showed no alteration.

The 10 salamanders examined on the 100th day had anaemic kidneys and their livers were very flabby. Under u. v. light the kidneys had a pale bluish fluorescence. The yellow points were not apparent.

Histologically necrosis and degeneration in the liver, and parenchym degeneration in the kidneys were found. The organs of the controls were normal.

Benzpyrene in olive oil solution. 7 salamanders were injected with 0.5 ml of a 0.5% solution of benzpyrene in oil, under the skin of the back. 3 were injected with the same quantity of pure olive oil.

The animals were examined on the 100th day. In the liver and kidneys the same alterations were seen as in those treated with the colloid solution. Tumour development was not observed here either.

THE EXPERIMENTS ON THE FROG.

Treatment by painting. 12 frogs were painted 3 times weekly on the abdominal skin with a 0.3% solution of benzpyrene in acetone for 3 months. 3 drops of the solution were applied. Before painting the skin was wiped dry; after the treatment the animals were kept in a dry place for half an hour or an hour. 3 controls were painted with pure acetone at the same time.

After the 3d—4th painting the skin began to peel and this went on till the end of the experiment. The skin analysed histologically showed the same effect, namely the peeling off of the epidermis on the painted area. Surrounding the painted area a normal regeneration



Fig. 1. Kidneys of a benzpyrene-treated frog in ultra violet light. Numerous small points in the kidney tissue show the characteristic yellow fluorescence of benzpyrene.

without proliferation was to be seen. The greenish yellow fluorescence of benzpyrene was noticed in the subcutis, in the glands, and in the remaining epidermis cells. The endothelium of the vein swelled and there were many erythrocytes. Peeling of the skin, and dilation of the vein was also seen in the controls. (So this is not the effect of benzpyrene but of acetone.) The yellow points on the kidneys were to be observed in u. v. light. (Fig. 1.) The urine of the animals had a yellow fluorescence too. The gall-bladder showed a blue light.

The histological picture showed a toxic haemorrhage surrounding the renal tubules. In the liver was a dissociation and a partial necrosis of the liver cells. The organs of the control animals showed no change.

Treatment with colloid solution. 23 frogs were treated with a serum-glycerine-benzpyrene solution injected 1 ml. under the abdominal skin 3 times weekly.

6 of the animals were killed on the 5th day, 10 on the 26th day and 7 between the 38th and 90th days. 5 control animals were treated similarly but without benzpyrene.

On the 5th day the decapitated frogs had a 2—3 ml. distinct opalescent exudate under the skin of the back and in the cavity of the abdomen with a greenish-yellow fluorescence. The exudate contained a few erythrocytes and some leucocytes.

In 3 cases myocardic hypertrophy was found. The structure of the lungs became loose, the fibres swollen and in the alveoles haemorrhage, peeled endothelial cells and exudative cells appeared. Haemorrhage occurred in the livers and kidneys too. Fluorescence microscopy showed the presence of benzpyrene in the cortex of the kidneys and in the endothelial cells of the renal tubules. The gall bladder had a blue fluorescence under u. v. light and the yellowish-green benzpyrene points were to be seen in the kidneys in only 3 cases.

10 frogs examined on the 26th day showed the same exudate as those of the 5th day. Hypertrophy of the heart was to be seen, and the kidneys were anaemic and showed the characteristic colour of parenchym degeneration. The livers had a greenish-brown colour and were flabby; the lungs were pale and also anaemic.

Under u. v. light the kidneys had a diffuse bluish colour with a few yellow points. This bluish colour was also found in the livers. The gall bladder had an ultramarine blue fluorescence.

In the section of the kidneys parenchym degeneration or degeneratio adiposa was noticed, as well as necrosis and toxic haemorrhage. The veins were full of erythrocytes. The structure of the livers was

dissociated, in some areas the liver cells were necrotized and in the intact areas the pigmentation of the liver was increased. The endothelium of the veins was defective. In 3 cases a special degenerative phenomenon was noticed, which was best demonstrated by MALLORY's stain method. Looking at the sections, areas of light blue colour can be seen, of 10—12 cell size, different from the bluish-red colour of the other normal areas. It is possible that in these marked light blue coloured tissue areas a chemical reaction takes place. In these marked areas are many pigment cells, and uany nuclear pyknoses and karyorhexes are to be seen.

The fibre of the lungs swelled, in the alveoli peeled endothelial cells appeared in the exudate. Benzpyrene fluorescence was to be seen only in the cortex and renal tubules.

On the 60th and 80th days 3 decapitated frogs showed changes in the lungs. The alveolar wall was voluminous and there were many cell accumulations similar to a tumour development process. But in this case it cannot be a tumour development because the cells have no malignant character. It seems to be a benign process caused by the exudate. (Fig. 2.)

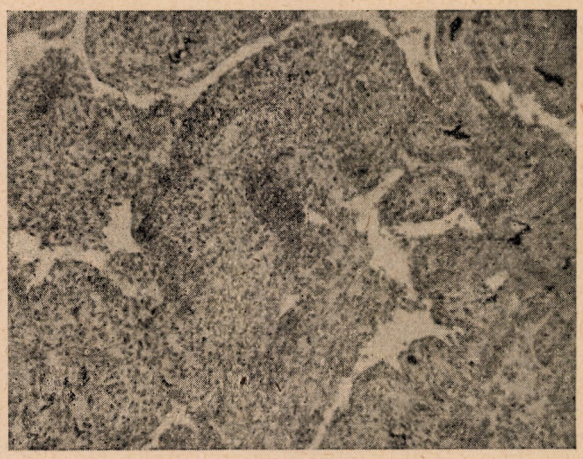

Fig. 2. Cell accumulations in the lung of a benzpyrene-treated frog.

In the other organs were changes similar to the previous series, except that here the light blue colour of the liver and kidneys under u. v. light was partial. The controls showed no alteration.

Treatment with benzpyrene dissolved in olive oil. 10—10 frogs were injected with 0.5 per cent solution of benzpyrene in olive oil. 0.5

ml was given in the adductors of the femur or under the abdominal skin. 4 controls received only pure olive oil, in the same quantity. The animals were examined on the 5th, 27th, and 70th days.

Tumour development was not observed, only a necrosis of the muscle fibre or strong hyperaemia of the skin.

Treatment with intravenous colloid solution of benzpyrene. 3 frogs were injected with 0.5 ml. serum-glycerine-benzpyrene solution intravenously. 12 hours later the strong yellowish fluorescence of the benzpyrene appeared in the kidney, the gall bladder had a weak blue fluorescence, kidneys and liver in sections showed haemorrhage.

24 hours later the fluorescence microscopic effect was the same as before,, except for the gall bladder, which had an ultramarine fluorescence.

DISSCUSSION.

To summarise, the effect of benzpyrene on amphibia is: Painting results in the epidermis cells absorbing benzpyrene but this does not cause tumour as is to be seen in the case of many mammals. Acetone causes peeling.

Subcutaneous, intramuscular, intravenous injection of colloid solution, and oil solution are absorbed in the organs and disturb the cell functions. The veins become more permeable, edema appears under the skin and in the abdominal cavity and in the lungs. The heart has to work more on account of the bad functioning of the veins and because of the haemorrhage of the organs. Thus hypertrophy of the heart occurs.

The degenerative and necrotic changes (in liver and kidneys etc.) can be taken as the known toxic effect of the cancerogenic hydrocarbons noticed in mice or other mammals *(Supniewski-Hano,* 1936, *Gaetani-Lanza,* 1937, *Maisin-Coolen,* 1936, *Parsons,* 1936, *Mauer,* 1931, *Wollbach,* 1937).

The various colours of the benzpyrene in the kidneys under u. v. light has been noticed. Benzpyrene has a greenish-yellow fluorescence in crystal form. Dissolved in colloidal solution it retains its colour, but in alcohol solution the blue colour appears. Higher temperature also changes the colour. (Weigert F., Mottram J. C., 1940). In our investigations it was to be seen that the kidney tissues adsorb benzpyrene and at first the benzpyrene in them has a yellow fluorescence. Later, when the tissue degenerates, it changes to blue. Thus physico-chemical

changes occur in the benzpyrene molecules during the degeneration of the kidney tissue.

It is to be mentioned too that in no case was fluorescence noticed in the depot fat of the frogs, though the great affinity of benzpyrene for fatty tissue is well known.

On the basis of our experiments we agree with F. DURAN-REYNALS, that benzpyrene does not cause tumours in salamanders and in frogs, but causes a very strong toxic effect in the cells and organs.

SUMMARY.

3—4 benzpyrene does not induce tumours in *Triton cristatus* and *Rana esculenta,* but it produces a toxic effect in their bodies. It produces a toxic haemorrhage in the kidneys and liver, parenchym degeneration, fatty-degeneration and necrosis. In frogs it produces edema in the lungs, exudative cells and an edemic swelling of the alveolar wall. Edema forms under the skin and in the abdomen. Benzpyrene is excreted by the liver and kidneys.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for the English translation.

REFERENCES.

DITTMAR (1939). *Protoplasma* **32.**
DURAN-REYNALS (1939). *Yale I. Biol. a. Med.* **11.**
GAETANI-LANZA (1937). *Arch. Ital. Med. Sper.* **1.** 21.
GRAFFI, H. (1939). *Zschft. f. Krebsforsch.* **49.** 477—495.
KOCH-SCHREIBER C. B., SCHREIBER G. (1939). *Bull. Assoc. franc. Etud. Canc.* **28.**
MAISIN, COOLEN (1936). *Compt. Rend. Soc. Biol.* 117—119.
MAUER, (1938). *Arch. f. exp. Zellf.* **21.** 191.
PARSONS, (1936). *Journ. Path. a. Bact.* **43.** 1.
SUPNIEWSKI, HANO (1936). *Bull. int. Acad. Pol. d. sci. et lett. Cl. med.* **43.**
WEIGERT, F., MOTTRAM J. C. (1940). *Nature* 895—896.

MICROSCOPIC INVESTIGATION OF MUSCLE FIBRIL TURNED ON ITS LONGITUDINAL AXIS.

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With 4 Figures in the text.

(Received for publication 1st May 1946.)

The smallest unit in the cross-striated muscle observable with light microscope is the muscle fibril, in which dark and light striations follow each other, that is to say, seen in polarized light, successive isotrop and anisotrop segments. Numerous investigations have attempted to reveal the structural cause of this optical cross-striation. In classical opinion it was caused by the fibrils' being made of two different materials which, making discs, alternated with one another.

But beside the classical opinion, the view has often been expressed that the fibril's striated appearance was not due to differences in substance but to an optical illusion resulting from the geometric placing of the sub-microscopic particles — the micels — which are part of the fibrils' construction (HÜRTLE, 1931).

In recent years SZENT-GYÖRGYI (1940—44) and his co-workers (I. BANGA, K. BALENOVIC, F. B. STRAUB) broke up the muscle's contractile substance into its 2 components, myosin and actin.

The union of the 2 substances according to stöchiometrically determined proportions creates the contractable actomyosin. On the basis of data on the components' physico-chemistry and observations from the standpoint of place-filling, mechanism and function, SZENT-GYÖRGYI (1946) attributes the spiral structure to acto-myosin.

As the spiral structure causes a certain periodicity in the substance's geometrical placing, it was natural to think that perhaps the cross-striation too could be brought in relation with the spiral structure of the actomyosin threads running through the fibrils. (SZENT-GYÖRGYI, l. c.)

The problem can be decided on the basis of the following considerations: If the fibril is made of discs then when it turns the cross-

striation would always have to stay in the same place. But if the spirals extend throughout the fibrils, then in turning, while the optical picture remained unchanged, the cross-striation would have to shift.

The turning of the fibrils was attempted by SZENT-GYÖRGYI, later by GERENDÁS, and it was established that when the fibril is turned the cross-striation does in fact move in a way corresponding to the above assumption. But, due to technical difficulties, it was not possible in a single case to follow the shifting of the cross-striation throughout the complete rotation of the fibril. Furthermore, the optical changes caused by the alteration in distance from the object (see below) were not taken into account.

In the present investigations we intended primarily to eliminate the changes in the microscopic image arising from microscopic depth of the fibril, furthermore, by developing the rotating technic, to follow the behaviour of the cross-striation throughout the fibril's complete rotation and to demonstrate it by microphotographs.

EXPERIMENTAL MATERIAL AND PROCEDURE.

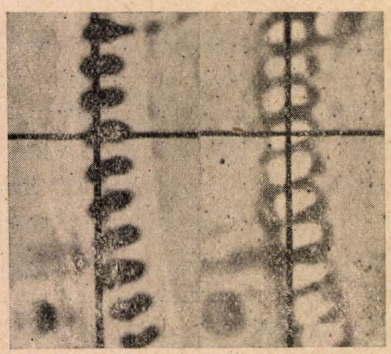
For the experiments the flying muscles of the thorax of a water beetle (Hydrophilus) were used. The preparation of the muscle was carried out after a method similar to SZENT-GYÖRGYI's. The flying muscle was fixed in 1% osmic acid, after which it was finely minced with a sharp knife. The minced muscle was shaken for 30 minutes in frog-Ringer solution with glass pearls, then centrifuged. On this mechanical interference the fibres separated into fibrils. The centrifuge was mixed with a 10% slightly warmed gelatine and then, while still lukewarm, sucked into a capillary glass tube. After the gelatine had stiffened the capillaries were microscopically examined and those selected in which single fibrils were centrally placed and their long axis parallel with the long axis of the capillary. For rotating the capillary a Zeiss capillary rotator was used, attached to the object table of the microscope. The area between the capillary and the lens was filled with cedar oil. This instrument assured that during the rotation the capillary made no excentric movement, it prevented the deplacement of the capillary along the axis, and its division in degrees made possible the constant control of the angle of rotation. The photographs were made with a Contax machine mounted on a (Reichert) microscope, with the addition of an observation apparatus consisting of a mirrorprism and side observation tubes. The photographs were made with an enlargment A=0,75, 60x

and 10 x photo-ocular. The most difficult task was to eliminate the movement of the different parts of this installation. We achieved great stability by fixing the microscope, the photographic appendages and the Contax apparatus on a steel rod resting on a wide base. With this the rotator could be turned, the film rolled and exposures made without the slightest movement of the contraption.

EXPERIMENTAL RESULTS.

a.) The change in the optical image due to alteration in distance from the object.

SCHÄFFER (1922) in his description of cross-striation of muscle fibre announces the phenomenon that if we move the lens of the microscope from a higher to a lower situation the Q striation changes places with the I striation. In our investigation we found the same appearance in the fibrils (Figure 1).



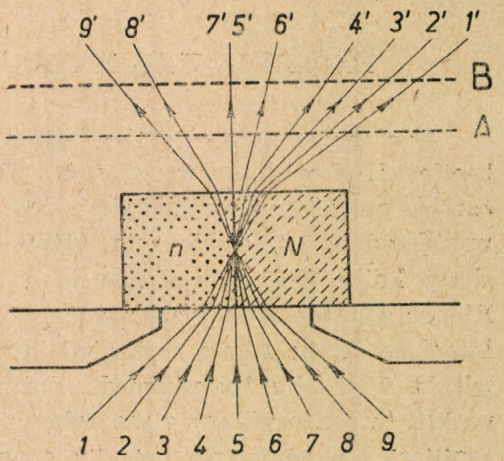


Fig. 1. An isolated wing-muscle fibril. Shifting of the cross-striation caused by moving the lens of the microscope to higher (A) or deeper (B) situation.
Fig. 2. Explanation of *Becke's* phenomenon.

The observed change of striation is to be explained by BECKE'S phenomenon (Figure 2). It is essentially that: The condensor of the microscope projects a conical light-bundle on the object. The path of the light-bundle is influenced partly by the reflection, partly by the refraction at the point of contact of the different refractive media of the object. Let the refractive index of the two media be n, N respectively, and $n < N$.

Investigating the surface of contact of the two different refractivable media through the microscope a part of the light rays arriving from the microscope mirror penetrate from the medium N into medium n, another part from n into N. In consequence of the total reflection the greater part of the light rays get into N medium, and only a smaller part continues to go through n medium. (8, 9) Those rays which reach the N medium at a greater angle than the limit-angle of the total reflection are reflected on the contact surface and remain in the same medium. (6, 7). Of the rays arriving from the N medium only those can penetrate in the n medium, whose angle of incidence is less than the angle of the total reflection. On the other hand, the rays reaching n medium from the microscope mirror can all penetrate into N medium. (1, 2, 3, 4).

For this reason the intensity of the light increases on the border of the N medium (the rays condense there), that is, there appears a narrow light stripe, BECKE's line.

When we move the lens of the microscope to a higher situation the light-stripe widens in the material which shows greater refractive index and it seems as if it moved to the interior of this medium.

In the case of the fibril, the anisotropic striation (Q) is the medium of greater refractive index, the isotropic striation of less. Moving the lens to a higher situation a light-coloured stripe begins to move from each side of the anisotropic striation towards its interior. These light-stripes cross each other at various heights and at these points increase the light intensity. (Figure 3). Thus, due to the crossing of light rays, the light stripes appear above the Q striation in situation A and above the I striation in situation B.

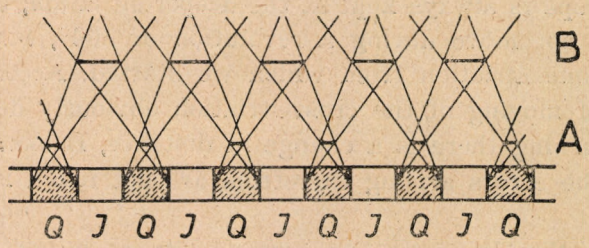


Fig. 3. Explanation of interchange of cross-striation on the basis of *Becke's* phenomenon.

In investigating the significance of this matter, when we wish to observe the shifting of the cross-striation, this circumstance may cause errors, for the fibril in the capillary, because of its excentric position, assumes in turning now a higher and now a lower position (with the

greatest care it is never possible to find fibrils in the capillaries which lie exactly parallel to the capillary axis) and thus the shift of the cross-striation itself can be caused by the difference in distance.

In our experiments we had in any case to make certain that in turning we did not change the distance from the object. We succeeded in this by always examining the fibril in the deeper situation (using the upper-situated ones as controls).

b.) Rotation.

To determine whether the fibril's cross striation moves or not it was necessary to take a fixed basic position to which we could return after the rotation. For this purpose we selected an I stripe beside which small fibril pieces could be seen. We adjusted the cross-thread from this known I stripe to the fourth I stripe without disturbing the particles in the process of the observation.

The rotation was accomplished in such a way that the cross-thread was kept on the I striation during the constant observation of the fibril picture, while the capillary-rotation drum was extremely slowly turned 2°. After each turn, as control, we turned to the higher situation to observe whether the cross-thread stood on the dark stripe. Every 10° photographs were taken.

After a rotation of 360° we returned to the marked basepoint and found the cross-thread on the same I stripe from which we started. (Figure 4.)

DISCUSSION.

The results of the experiment show that if single fibrils of water-beetle's flying muscle are turned round their long axis and the lens of the microscope is always kept at the deeper situation, then the cross striation does not move during a revolution of 360°, but that we return to the same striation from which we started. According to this, observations of moving of the cross-striation obviously derive from the fact that in turning there is a change in distance, BECKE's phenomenon appears and the errors arising therefrom are not taken into consideration.

According this basis, in our experiments at every angle in the revolution we should have got an image of exactly the same character. If we look at the photographs (Figure 4) we can observe consistent changes in the shape and size of the Q and I stripes. In these changes we find regularity if we begin with those photographs in which the I stripes are the largest (at about 130°). At 180° from this point (about 310°) the Q-stripes are the biggest, then in the pictures at 90° from

either, (at about 220° and 40°) the Q and I stripes are equal size. On the basis of observation of the pictures it is our impression that the fibril is not symmetrical but that its front, back, right and left sides could be distingushed and the photographs obtained of this could be the optical demostration of an assymetric structure. It is possible that this refractive assymmetry is caused by the submicroscopic structure of the fibril.

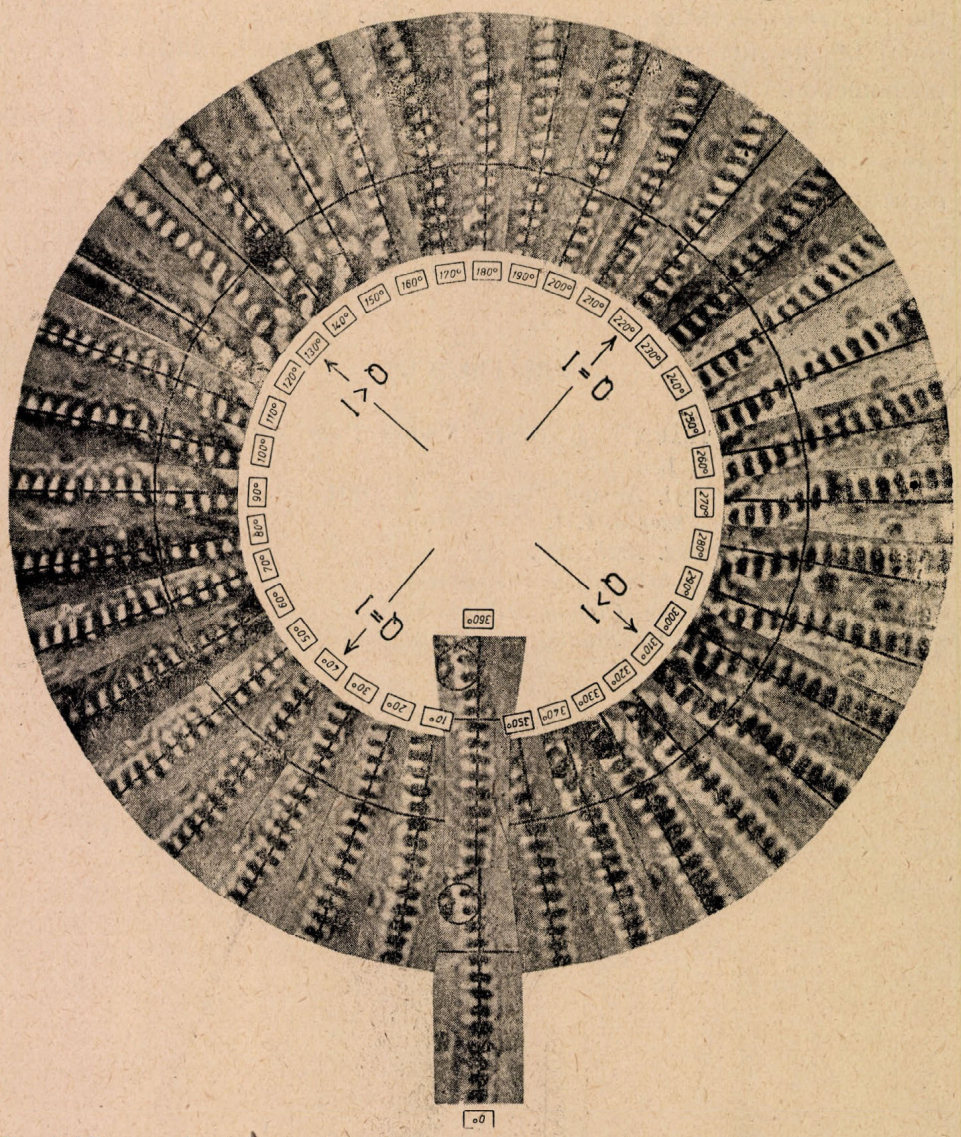


Fig. 4. Microscopic pictures of an isolated wing-muscle fibril of Hydrophilus turned on its longitudinal axis.

SUMMARY.

Microscopic investigation of the fibril of water-beetle's (Hydrophilus) flying muscle established that the cross-striation of the fibrils moves because of changes in distance from the object. Taking this into account, we turned single isolated fibrils identically distant. The cross striation of the fibrils did not move during rotation. According to this the former observations of movement in the cross striation took no account of the differences in distance from the object and of BECKE'S phenomenon.

Photographs taken every 10° of rotation show that the fibril's cross-striation is due to assymmetry in the material's submicroscopic structure.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for the English translation.

REFERENCES.

BALENOVIC, K. and STRAUB, F. B. (1942). *Studies from the Inst. of Med. Chem Univ. Szeged.* **2.** 1.

BANGA, I. Szent-Györgyi, A. and Straub, F. B. (1941—42). *Studies from the Inst. of. Med. Chem. Szeged.* **1.** 1.

HURTLE, K. (1931). *Pflüg. Arch.* 227.

SCHÄFFER, J. (1922). *Lehrbuch der Histogenese.*

SZENT-GYÖRGYI, A. (1946). *Colloid Science.* **1.** No. 1—19.

SZENT-GYÖRGYI, A. (1944). *Studies on muscle. Szeged.*

THE QUESTION OF THE EQUALITY OF SOMATIC AND GERM NUCLEI IN RESPECT TO HEREDITY AND SURVIVAL, ON THE BASIS OF STUDIES IN A SOIL PROTOZOON.

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With 15 Figures.

(Received for publication 1st August 1946.)

From MAUPAS (1886) and R. HERTWIG (1889, 1914) we know that Ciliata protozoa have two sorts of nuclei: somatic and germ nuclei. Furthermore, that the role of the somatic, or macronucleus is the preservation of the cells and vegetative propagation by amitotic division. The germ nucleus, or micronucleus, goes over by mitotic equatic division into the vegetatively propagating successors. But, as HERTWIG and others have established, it keeps the potentiality that during sexual reproduction the reduced haploid germ nucleus, which we call the micronucleus, reciprocally unites with the germ nucleus of its conjugating partner and recreates the somatic or macronucleus which has disintegrated. WOODRUFF (1921) and others after him established the same process in the course of endomixis or parthenogenesis. This fact was established in the beginning simply by observation, but later various authors confirmed it experimentally, removing the germ nucleus and observing in the presence of the somatic nucleus alone the vegetative and propagative life-functions of animals. (CALKINS, 1930, DAWSON, 1919, 20, PATTEN, 1921, WOODRUFF, 1921, SCHWARTZ, 1939, TZE-TUAN CHEN, 1940, REYNOLDS, 1938, PIEKARSKI, 1939, KIMBALL, 1941.) From the foregoing it appeared that the somatic, or macronucleus itself alone could assure the entire life function, with the exception of sexual reproduction and parthenogenesis. In this case (i. e., sexual propagation and parthenogenesis) the somatic nucleus is destroyed and, the germ nucleus not being present, there is nothing with which to re-build and so the animal dies. In reality, therefore, these experiments seemed to confirm the theory of nuclear dimorphism founded by R. HERTWIG: the macronucleus as somatic nucleus disappears without leaving a trace,

but the micronucleus is deathless, as according also to WEISMANN (1892).

In my experiments, as follows, I propound that, contrary to existing opinions, the somatic nucleus is equal to the germ nucleus in a soil cilia protozoön in respect to heredity and survival when the micronucleus has been experimentally eliminated from the organism.

CHOICE OF EXPERIMENTAL OBJECT AND EXPERIMENTAL TECHNIC.

For experimental purposes a 100-micron-sized soil protozoön (the hypotrichous *Kahlia simplex* HORVÁTH 1934, 1935—36) is used. These animals have 2 macro and 2 micro nuclei. (In reality more are demonstrable, but only 2 can function.) They are easily cultivated in a water extract prepared from a soil in which the microorganism lives. The simplest method of preparing is by adding 1 litre water to ½ kgm. soil, shaking it several times, 12 hours later filtering it through a filter paper, then to the filtered solution adding 0.3 g. gelatine and dissolving it warm. The animal is in the best condition for cultivation when it divides 4 times daily; i. e., when it has produced 16 progeny.

My aim was to find an easy method of eliminating the micronucleus from the animal's body. For that ultra-violet rays brought about the desired result. The procedure was as follows: The animals being in about a 5 ml. food solution in a Boveri cup, with a Hanau quartz lamp I directed a just less than lethal dose of u. v. rays upon them. The animals reacted variously to the rays. A relatively small percent of them were utilisable for our purposes. The effect of the u. v. rays on a small percentage of them was to create twin-figures, joined at the back. These twin forms made possible the creation of animals containing only the macronucleus. (I refer you to the work of my colleague F. ÖRDÖGH (1941), to whom I gave this problem for detailed study, while for myself I kept only the part dealing with the role of the somatic and germ nuclei.)

The real cause for the formation of the twin figures it was not possible to establish. According to our observations they could be formed in the following way: A small percentage of the animals were submitted to the effect of the u. v. rays as they moved about on the bottom of the vessel, which meant that the back or the ventral surface of the animal was more strongly exposed to the rays, as our animal has a dorsi-ventral flattened body. The reversibility of the coagulation brought about by the u.-v. rays showed the latest in that part

of the body which was directly touched by the rays. Thus could arise the interesting situation that the organisms, more quickly overcoming the effect of the u.-v. rays in the vicinity of the back or ventral surface, could there begin locally and gradually to grow. As effect of this one-sided growth the animal takes a V-form, as can be seen from drawing. (Figure I. a.) This state occurs 3—4 hours after the u. v. radiation. The two sides of the V in reality each represent an animal which appears as an abnormal division. Mouths develop at the two ends of this V. Then, as result of constant one-sided laming, the two individuals which were disturbed in the process of division close almost together. The division following this state definitively created the twin figures joined at the back. It is possible that the 2 individuals originating from the front part can again take a V-shape, but those originating from the back already have a form as if they were complete mirror-pictures of one another. In movement the two behave as one animal, but from the standpoint of organization as two, having 2 adoral zones, 2 motile organs, 2 mouths, 2 cytopyge, 2 contractile vacuoles, and double sets of nuclei. ÖRDÖGH and I found that this twin development is only a lasting modification which could be maintained only with optimum cultivation and nutrition for any length of time. If the cultures, kept at room temperature, were not transferred every 3—4 days then the separation of the animals took place. If over a longer time the culture was not refreshed all the animals separated. This phenomenon could be used in the elimination of the germ or micronucleus. That is to say, in the longitudinal separation — as could be seen from the coloured preparations — the micronuclei did not disperse regularly so that they necessarily arrived in both halves. If now we cultivated the separated individuals separately we formed of them the so-called clon, and could shortly see in the coloured preparations from which clon the micronucleus was missing.

OTHER TECHNICAL PROCEDURES.

I made good use of a variety of aceto-carmine staining of the nucleus which I had earlier worked out (1940). The microphotograms were also made from preparations staining in the same way. I used FEULGEN'S nuclear reaction as standard preparation.

THE VEGETATIVE FUNCTION OF AMICRONUCLEATE ORGANISMS.

In general the amicronucleate organism does not differ in its exterior morphology or appearance from the normal animal. From the

standpoint of motion, nutrition, digestion and excretion, as well as osmotic conditions, they also seem identical. Similarly, the progeny produced in a given time in the course of propagation by division in 2 is also numerically the same as in normal animals. To this extent it corresponds with the findings of others (DAWSON, PATTEN, WOODRUFF). But there is a difference from the standpoint of life-span. After a certain number of generations, according to previous experiments, the amicronucleate Ciliata die out.

In my experiments this dying-out does not occur. I have cultivated the animals through 1800 generations up to now and left the cultures encysted, but I can excyst them at any time. In itself the circumstance that they are capable of normal cysts makes possible the continued existence of these amicronucleate animals.

PARTHENOGENESIS or endomixis occurs from time to time (on an average of every 8—10 days) in our animals too. The dimensions of the animal's body diminish, the somatic or macronucleus breaks up into pieces. On the basis of WOODRUFF's experiments we should expect that the somatic nucleus would be absorbed and, there being no germ nucleus and therefore nothing from which to build up anew, that the animal would die. After the disintegration of the nucleus which is associated with such a diminution of body-dimensions our amicronucleate animal begins again to form the macronucleus out of the stumps of the old one if we transfer it to a new culture. The individuals which have gone through parthenogenesis and which have been transferred to fresh ground regain their normal capacity to divide in 48 hours. We can also bring about parthenogenesis artificially by giving them a greater quantity of bacteria as nutriment. If we give the same amount of bacteria immediately after parthenogenesis they do not react as quickly with repeated parthenogenesis (small body-size, disintegrated nucleus) as before. On the contrary, the body remains big for 72 hours. Shortly their bodies conform to those of animals with normal nuclei living under optimal conditions, in spite of the effect of the great amount of bacterial metabolites. This resistance may be attributed to the regeneration due to parthenogenesis.

There is however a fairly rare phenomenon in regard to the somatic nucleus, first mentioned by HERTWIG, which seems to be catastrophic for the animal. That is, the body of the animal becomes smaller in this case too, but this is not followed by the disintegration of the macronucleus. First both parts of the macronucleus grow excessively in comparison to the size of the animal's body. The growth can be such that, not finding sufficient room within the body, its ends roll up.

In that phase the large nucleus is still rich in chromatin granules and does not differ in its inner structure from the macronucleus in normal animals. 50—60 hours after this phenomenon takes place the large nucleus begins gradually to swell up, becomes hollow, then falls to pieces. At the same time the body becomes round, and the animal dies in 7—8 days.

CONJUGATION.

The amicronucleate Kahlias conjugate relatively easily if the temperature of the culture is elevated to 30—35°. As can be seen from the first microphotogram, conjugation begins by the first conjugating pair adhering at their frontal parts. (Fig. 1.) The 2 individuals of the conjugating pair are always of different size; that is, they couple by anizogamic conjugation. At the very beginning of conjugation the macronuclei lose their regular oval shape and become irregular in form. In the next phase, as can be seen from microphotograms 2 and 3, the macronuclei become elongated in fronto-caudal direction and develop a long ribbon-like shape. The macronuclei of normal animals show in this stage of conjugation a more advanced development, as is to be seen in the 4th microphotogram: The macronuclei begin to fall to pieces in inequal divisions and at the same time the chromotin granules begin to be absorbed in the plasma.

A further stage of conjugation is shown in microphotogram 5: The conjugating pair become fused in their whole length, so that the tails of the 2 animals can be seen only in the caudal part; the macronuclei fall by division into several parts. As is shown in the 6th microphotogram, the smaller of the conjugating pair gets fused with the body of the bigger one to such an extent that it is seen only as a swelling. At the same time the macronuclei multiply by further divisions, but simultaneously some of them begin to disappear in the plasma.

The total fusion of the conjugating pairs is not characteristic for normal animals.

In other Ciliata and in normal *Kahlia* the next conjugating phase can be seen only after the coupling of the germ nuclei and after the separation of the pairs. In our amicronucleate animals, however, there are no ex-conjugants, and therefore this technical term cannot be used; we are obliged to continue to speak of conjugation. This state stops only, not alone when the 2 conjugants look to be one from the outside, but when their nuclei are reduced to the set of nuclei of a single animal. Our amicronucleate animal is shown at this stage in microphotogram No 7:

The macronucleus is doubled, but this is not yet a vegetative form even though it may seem to be, for one of the macronuclei is in process of absorption. Its borders are corroded, bubbles appear on its body, these two things being indications of absorption. That equilibrium is not reached at that stage is proved by the 8th microphotogram: Though the 2 greater macronuclei are in the process of absorption, as is shown by the corrosion of their edges, 2 new somatic nuclei of the size of a micronucleus appear at their sides. In the next phase there are no more big somatic nuclei but in their place we find 2 or 3 nuclei of the size of the micronucleus (9th microphotogram). Following this the 2 permanent macronuclei are formed (10th and 11th microphotograms). The successful end of the conjugation is shown on the 12th diagram, when the animal begins to divide. The nuclei are fused and begin to close in at the middle, so as to be able to divide into the 2 successor cells.

Conjugation takes place on an average in 2—3 days. During this time our animals take no nourishment, and so the symptom occasionally occurs that the body diminishes decidedly. At such times the animal loses its roundness and, though it develops again in the final macronucleus form, it cannot reorganize its body, and dies. The changes which such a fate after conjugation cause in the ratio of nucleus to cytoplasm can be seen in the 13th and 14th microphotograms. The resistance of the conjugating individuals varies enormously and for that reason it is never possible to give averages for the mortality arising in consequence of conjugation. I have had experiments where of 10 pairs not one died, while there were others with a death rate of 50%.

DISCUSSION.

Our findings coincide with those of former experiments in this, that amicronucleate Ciliata are capable of vegetative functioning, including also division. (In my earlier experiments (1939) the deaths were probably due to the operating technic not being suitable.) Here the question also arises as to what age the Ciliata propagating only vegetatively can attain. According to previous experiments (Dawson, Patten, Woodruff, *et al.),* after certain varying numbers of generations the extinction of the culture takes place. In my experiments there is no question of such extinction, under normal cultivating conditions, but there are cases if the large quantity of bacteria affects the protozoa. But at such times not only the amicronucleate individuals die out but also the control Kahlia. So this can be taken as a

pathological condition, and does not agree with JOLLOS' ideas on the length of life of the amicronucleate animals, which establish that they must die out because the macronucleus is absorbed during parthenogenesis and, there being no micronucleus, the animal cannot replace the loss. In our experiments it is proved that during parthenogenesis the macronucleus is capable of regenerating of itself. This gives the means of judging the results which can be expected from sexual reproduction (conjugation) too. (Presuming that in respect to the macronucleus parthenogenesis (endomixis) has the same value and role as in conjugation). Those authors (SCHWARTZ, TZE-TUAN CHEN, KIMBALL) who could make the amicronucleate Ciliata conjugate all of them report that conjugation is a catastrophe in the life of the amicronucleate animal, because the macronucleus is absorbed (as in endomixis) and the animal without nucleus dies.

In my experiments I proved that the macronucleus regenerates after conjugation. My observations, made during the conjugation of the amicronucleate animals, was that the macronucleus regenerates from the parts of the macronuclei which have the same size and structure as the micronucleus; and this led to the supposition that in normal conditions the macronucleus does not derive from the micronucleus, but should regenerate independently, of itself. If this were the case then we should have to speak, not of nuclear dimorphism but of double nuclei. Beyond the proof of change of form which can be seen in the microphotograms we know from our experiments with the macronucleus too that it assures itself the functions of reproduction and survival. The contrary of this experiment, that is, that only micronuclei should be present in the animal, would decide whether the micronucleus were equivalent to the macronucleus in the vegetative life-span also. As I did not make such experiments it can be taken as only hypothetical that the somatic role of the 2 nuclei are identical. Experiments in polyploidizing the micronucleus (MACDOUGAL, 1931) prove that the micronucleus is the germ nucleus, and my experiment proves the identical qualities of the micronucleus with the macronucleus in sexual reproduction.

How do we reconcile the differing findings of previous experimenters with our own?

We might suppose that the earlier experimenters had worked under an error to which could be ascribed the differences in results. The conjugation of our experimental animals is a peculiar conjugation, differing so much from the characteristics of the object in the similar experiments of others that one is inclined to say that there is no analogy

between my experimental object, the *Kahlia simplex,* and experimental objects of the others, but that they differ.

If the others' experimental error is the cause of the discrepancy in our experimental findings, then the previous theory of somatic and germ nuclei division is entirely overthrown. If in our experiments we have to deal with an extraordinary object then there is no ground for generalizations about rules, for most probably our experimental object is not a unique exception. Thus the previous conceptions of nuclear dimorphism can be refuted ,as the nucleus called somatic can perform the same functions as the germ nucleus. It can even be included in WEISMANN's conception of immortality. Therefore clearly not nuclear dimorphism but double nuclei are in question.

The entire experimental findings cause serious difficulties only insofar as we do not know where in the somatic nucleus to place the genes of heredity. In his studies of conjugation KITZKE (1916) saw chromosomes in the growing macronucleus which were covered as they developed by increasing amounts of chromatin granules. IVANIC (1933) and PIEKARSKI (1941) also reported sorts of Ciliata in which there might be chromosomes in the macronucleus. But we did not find this in our animals. On the other hand, the fundamental beginning of reorganization of the somatic nucleus after the conjugation of amicronucleate animals is formally identical with the macronucleus in a resting state. According to WEISMANN we should take this as the germ part existing in the somatic nucleus, because it regenerates the somatic nucleus. It is to be supposed that the genes of heredity are also therein.

SUMMARY.

1. From the *Kahlia simplex* HORVÁTH soil protozoön the micronucleus was eliminated by u. v. radiation.

2. From the amicronucleate *Kahlia* were established:
   a) The vegetative life-function capacities — digestion, selection, excretion, regeneration, division, encysting and excysting.
   b) That the life-span under optimum conditions was interminable.
   c) That the animals are capable of endomixis (parthenogenesis) and of conjugation.

3. In the amicronucleate *Kahlia* during parthenogenesis the macronucleus at first breaks up but under optimum conditions regenerates anew.

4. If during the conjugation of amicronucleate *Kahlia* the macronucleus breaks up and is absorbed to such an extent that only micronucleus-sized pieces are left, it can still reorganize from these.

5. Under points 2, 3 and 4 we can declare that the macronucleus is equal in value to the germ nucleus.

6. The genes of heredity are probably present at the inception of the macronucleus.

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REFERENCE.

GALKINS, G. N. (1930). *Arch. Protist. K.* **69.** 151.
DAWSON, J. A. (1920). *J. exp. Zool.* **29.** 473 and **30.** 129.
HERTWIG, R. (1889). *Abh. d. K. Beyer. Acad. Wissensch. München.* II. Kl. **17.** 1.
HERTWIG, R. 1914). *Biol. Zlb.* **34.** 557.
HORVÁTH, J. (1934). *Acta Biol. (Szeged).* **3.** 60.
HORVÁTH, J. (1935—36). *Arch. Protist. K.* **86.** 482.
HORVÁTH, J. (1940). *Z. wiss. Mikr.* **57.** 168.
HORVÁTH, J. (1939). *Állat. Közl.* **35.** 62.
IVANIC, M. (1933). *Arch. Protist. K.* **80.** 1.
KIMBALL, R. F. (1914). *J. exp. Zool.* **86.** 1.
KITZKE, M. (1916). *Arch. Protist. K.* **80.** 1.
MAC DOUGALL, M. S. (1931). *J. exp. Zool.* **58.** 229.
MAUPAS, E. (1886). *C. R. Acad. Sc. Paris.* **102.** 1562.
ÖRDÖGH, F. (1941). *Az ibolyántúli sugár hatása a Kahlia simplex Horvathra. Dokt. ért. Kolozsvár.*
PATTEN M. W. (1921). *Proc. Soc. Exp. Biol. a. Med.* **18.** 188.
PIEKARSKI, G. (1939). *Arch. Protist. K.* **92.** 117.
PIEKARSKI, G. (1941). *Biol. Zlb.* **61.** 416.
REYNOLDS, J. M. (1938). *Biol. generalis (Vienna).* **14.** 325.
SCHWARTZ, V. (1939). *Naturwiss.* **724.**
TZE-TUAN CHEN, (1940). *J. Heredity.* **31.** 185.
WEISMANN, A. (1892). *Über Leben und Tod. Aufzätze über Vererbung. Jena.*
WOODRUFF, L. L. (1921). *J. exp. Zool.* **34.** 328.

EXPLANATION OF THE FIGURES.

I. a) V-shaped double figure formed by ultra violet radiation.

I. b) Twin-form united at the back, derived from the V-shaped form. The double sets of nuclei distinctly visible. (Sketches after carminacetic acid staining.)

1., 2. and 3. Conjugation of amicronucleate animals. The different figures show the progress of conjugation: the micronucleus gradually prolonged. Microphotogram after carminocetic acid staining. The same method applies to the other, following, Figures.

Magnified: 240 x, 330 x, 210 x.

4. Conjugation of animals with normal nuclei Magnified 330 x

5. and 6. Further progress of amicronucleate animals' conjugation. Gradual fusion of the 2 bodies and the disintegration of macronucleus. Magnified: 240 x, 180 x.

7, 8, and 9. The more important phases of final formation of macronuclei. Magnified: 240 x, 250 x, 210 x.

10, 11 and 12. The definitive form of normal macronuclei and their first division. Magnified: 210 x, 240 x, and 180 x.

13 and 14. The new-formed macronucleus not followed by body growth. Magnified: 210 x.


a
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THE DISTRIBUTION OF THE DIFFERENT PROTEIN FRACTIONS IN NORMAL AND HYPERTROPHIZED CATS' HEARTS.

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(With 6 Tables in the text.)

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While trying to work out the mechanism of heart hypertrophies (HAJDU and BEZNÁK, 1944) the question arose of how the composition of the heart muscle itself changes during hypertrophy. Several authors have proved that there is a certain connection between the activity of an organ and its bio-chemical composition. WEBER and MEYER (1933) found that the distribution of the N, resp. protein fractions is different in white and red muscles. HITCHINGS and WEARN (1944), working on the distribution of intra- and extra-cellular electrolytes, found in rabbits that the extra-cellular electrolytes increased during the first few days, after the lesion of the aortic valves, but on the 6th day — that is, when hypertrophy was already present — the distribution became normal. Several authors proved the connection between activity and phospholipid content. Thus MAYER and SCHAEFFER (1914) in the case of liver and lungs, BLOOR, OKEY and CORNER (1930), KAUFMAN (1927) in the case of the active corpus luteum, BLOOR (1934) in the case of the mammary gland, MASUDA and BLOOR (1932), BIERICH DETZEL and LANG (1931) in the case of fast-growing tumors and BLOOR (1927) in the case of muscle. According to BLOOR and SNIDER (1934) the cholesterol content of working muscle also increases.

LUDEWIG and CHANUTIN (1936) found, contrary to this, no increase in the phospholipid content of hypertrophized rats' hearts counted per unit surface, nor did they find a difference in the creatin concentration

of these hearts (LUDEWIG and CHANUTIN, 1936). We were particularly interested in the protein content of hypertrophized heart muscle, resp. the distribution of protein in different fractions. Protein being the contracting substance of muscle, we wanted to find out whether the protein content or its distribution in different fractions changes during hypertrophy or whether there is in the course of the hypertrophy a phase in which a difference can be found.

METHODS.

Our experiments were carried out on cats of both sexes weighing 3.2 ± 0.6 kg. The cats lived on a mixed diet. In the first part of the experiments we carried out determinations on normal cats' hearts. The chest and pericardium of the animals were opened in Pernocton anaesthesia (0.8 ml/kg.). The heart was cut from the big vessels and perfused with ice-cold Ringer solution from the coronary arteries. The heart was then quickly freed from its pericardium and fatty tissue and the two atria were cut off. The right ventricle was cut along the septum and both parts (right ventricle, left ventricle+septum) frozen with CO_2 snow and weighed separately.

The lesion of the valves was carried out in ether anaesthesia by pushing down a long, round-headed rod through the left carotid artery into the ascending aorta and tearing at least one, possibly two, valves to pieces. The animals soon recovered after the operation and during the first week did not differ from normal ones. In the course of the second and third weeks some of the animals (in which as was later proved the valvular lesion was greatest) showed some slight signs of dyspnoe and avoided walking about much. In the course of our observation (30 days) we never saw serious decompensation (edema, cyanosis). We controlled the heart by taking electrocardiograph records; one from the heart before, one immediately after the valvular lesion, and one at the time when the heart was used for determinations. At different intervals the hearts of the animals were treated in the above described manner in Pernocton anaesthesia.

Determinations were always carried out on the part of the heart containing the left ventricle+septum. This was finely ground in a small Latapie mill and its total N-content estimated by simple micro-Kjeldahl method. From the remaining ground tissue the protein was then extracted. This was carried out by a slight modification of the WEBER and MEYER method (l. c.). The difference consisted mainly in employing

smaller amounts — since working with heart muscle we could not use as much tissue as WEBER and MEYER did. The extraction was carried out on 3.7 ± 0.8 g. ground heart muscle. The course of the extraction was the following: 5 extractions with 50—50 ml. of WEBER I solution, 6 hours each. The extraction was carried out according to WEBER at 0 C° temperature. The total time of extraction was 40 hours. In this way the N-fractions of the heart muscle were divided into two parts: one soluble which was in the 450 ml. of united WEBER I and WEBER II solutions, and one insoluble remaining in the muscular residue after the extraction.

In the soluble part we determined the non protein-N content by precipitation with sulphosalicylic acid. The separation of the proteins took place according to WEBER into myosin, globulin and myogen fractions. The myosin fraction was precipitated by dyalising through a cellophane membrane at 0 C° for 24 hours with constant shaking against a m/130, pH 6.3 phosphate buffer solution. The precipitate was centrifuged and its N-content determined. To 50 ml. of the myosin-free solution 4 times 25 ml. of a m/90 and pH 5.0 acetate buffer solution was added with constant shaking, then the whole was put into an ice-chest and shaken at 0 C° for an hour. The precipitate — corresponding to the globulin fraction — was then centrifuged and its N-content determined. The remaining solution containing the myogen fraction — soluble in these circumstances — was evaporated and its N-content determined. All N-determinations were carried out by simple micro-Kjeldahl analysis. In this way the soluble N-content of the heart was divided into 4 fractions: Non protein-N, myosin-, globulin-, and myogen-N.

The other part of the heart, that is the part remaining after the extraction, was treated in a way described by SPENCER, MORGULIS and WILDER (1937). The extracted muscular residue was dried with acetone and autoclaved for 3 hours at a pressure of 7 atmospheres. After centrifugation we determined the N-content of the remains, that is the fraction insoluble even under these circumstances, and called it Remaining-N. In the solution after centrifugation we precipitated the collagen and determined the N-content of the precipitate (collagen-N). The N-content of the solution after precipitation was also determined and called fluid-N. All N-determinations were carried out by simple micro-Kjeldahl procedure.

EXPERIMENTAL RESULTS.

Before describing our experimental results, we must say a few words about the experimental error of the methods used in the course of this work. We made 3 parallels to determine the N-content of the hearts. After the WEBER extraction the N-content of the extract was determined on 3 samples. The non protein-N was also determined on 3 samples. The determination of the myosin, globulin-, and myogen-N was made on 2 parallels in such a way that 100—100 ml. of the myosin extract was placed in two exactly similar dyalisors. After the myosin precipitate was centrifuged off 25—25 ml. of each was used for the precipitation of the globulin fraction; and the N-content of the two remaining fluids was also determined (Myogen-N). Of the non-extractable, acetone-dried muscle residue 3 samples were autoclaved; thus we had 3 parallel determinations of the remaining-, fluid-N, and collagen-N. The experimental error of these determinations is shown in Table I.

TABLE I.

Experimental error of the methods employed.								
Heart-N.	Extractable-N.	Non protein N.	Myosin-N.	Globulin-N.	Myogen-N.	Collagen-N.	Remaining-N.	Fluid-N.
± 1·8 %	± 1·5 %	± 2·3 %	± 4·1 %	± 8·8 %	± 6·8 %	± 5·5 %	± 12·1 %	± 6·9 %

Table II contains heart weights of cats whose aortic valves had been destroyed 9—29 days previously. Table II. shows that the hearts

TABLE II.

		Heart weight in g.			Heart weight in g. per kg. body-weight.			Left ventricle + septum weight in g.			Left ventricle + septum weight in g per kg body weight.			Left ventricle + septum per right ventricle		
	No	M	± μ	k	M	± μ	k	M	± μ	k	M	± μ	k	M	± μ	k
Normal	12	9·2	1·75	2·8	3·15	0·44	2·1	7·1	1·5	3·4	2·4	0·44	3·8	3·6	0·6	3·4
Hypertrophized	13	11·5	2·5		3·85	1·05		9·3	1·9		3·5	0·94		4·6	0·9	

of cats hypertrophize during that time and that this hypertrophy relates chiefly to the left ventricle. Table III. shows that the dry matter content of the heart does not change during hypertrophy. The content in dry matter is the same in the right and left ventricle whereas —

TABLE III.

Dry matter content of the hearts in %.								
Normal left ventricle + septum.			Hypertrophized hearts. left ventricle + septum.			Right ventricle.		
No	M	± μ	No	M	± μ	No	M	± μ
9	21·3	1·1	8	22·1	0·9	8	22·1	0·9
	→ k = 1·8 ←			→ k = 0·0 ←				

as seen in Table II. — only the left ventricle hypertrophized. Table IV. shows the N-content of the left ventricle in %. The hypertrophized

TABLE IV.

N-content of left ventricle + septum in %						Extractable N as % of the total N content					
Normal			Hypertrophized			Normal			Hypertrophized		
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
11	2·76	0·13	12	2·96	0·11	8	68·2	5·6	9	67·6	3·0
	→ k = 4·0 ←						→ k = 0·3 ←				

left ventricle contains somewhat more N than the normal one (significant difference 4.0). This surplus N seems to be divided very evenly between the different N-fractions for — as seen in following Tables — we found no difference between the N-fractions in normal and hypertrophized heart muscle. It is also to be seen from Table IV. what percent of the total N-content of the heart muscle can be extracted by the described method. In this respect normal and hypertrophized muscles do not differ. Table V. and Table VI. show the distribution

TABLE V.

The distribution of the extractable N into different fractions in %.																				
Myosin- N.				Globulin- N.				Myogen- N.				Non-protein- N.								
Normal			Hypertrophized			Normal			Hypertrophized			Normal			Hypertrophized					
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
8	22.5	3.4	9	26.5	7.8	8	12.2	4.3	9	11.5	5·3	8	13.8	3.4	9	18.4	8.0	8	10.7	2.8	9	17.4	4 5
	→ k = 1.4 ←					→ k = 0.3 ←						→ k = 1.6 ←						→ k = 0.4 ←					

TABLE VI.

The distribution of the non-extractable N into different fractions in %.

Collagen- N.						Fluid- N.						Remaining- N.					
Normal			Hyper-trophized			Normal			Hyper-trophized			Normal			Hyper-trophized		
No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$
8	15·7	2·7	9	13·4	1·5	8	9·4	2·7	9	11·1	2·9	8	7·5	2·2	9	5·0	0·9
	→ k = 2·1 ←						→ k = 1·2 ←						→ k = 2·8 ←				

of the different N-fractions. There is no difference between normal and hypertrophized muscle. Perhaps the remaining-N — that is, the part which is insoluble in WEBER solutions and ever after autoclave treatment — decreases somewhat in hypertrophized heart-muscle (significant difference 2.8).

DISCUSSION.

In the first part of our experiments we determined the protein fraction of normal cats' hearts. In 3 cases we also estimated the different protein content of the psoas muscle of the cat. The N-content of the psoas muscle was always more ($3.46 \pm 0.13\%$) than that of the heart ($2.76 \pm 0.13\%$).A larger portion of this greater amount of N could be extracted according to WEBER ($81.0 \pm 4.9\%$), whereas from the heart muscle only $68.2 \pm 3.6\%$ of its N-content could be extracted. Similar results were obtained in the case of skeletal muscles of rabbits by WEBER and MEYER (l. c.) who found that in the case of white muscles 85%, in the case of red muscles 76% of their respective N-content could be extracted. There is accordingly a certain difference in the extractability of the N-content of different muscles (white muscle, red muscle, heart muscle). DEUTICKE (1926, 1930, 1932) found that more or less protein could be extracted from frog muscles according to the state of the muscle in question. If we assume that the different N-fractions of the cats' skeletal muscles are about the same as the values given by WEBER and MEYER in the case of rabbits, we find the following differences between the N-fractions of heart and skeletal muscles: the heart muscle contains less extractable N, accordingly more stroma-N. The non protein-N fraction is greater in the heart muscle than in the skeletal muscle, and the myosin-, globulin-, and myogen-N (especially the myosin fraction) is less. As SZENT-GYÖRGYI and his collaborators have proved (1941)

that the myosin is only extractable in the presence of adenosintriphosphate, we first thought of the possibility that the heart muscle contained less adenosintriphosphate or one that was destroyed faster and in consequence less myosin could be extracted. Therefore we performed the following experiment: in 3 cases we divided the heart muscle: one part was extracted in the usual way, to each of the third to ninth extractions of the other part we added 5—5 mg adenosintriphosphate. There was no difference in the amount of N extracted. That we find less myosin-N in the heart is therefore probably due to the fact that the heart muscle contains a smaller amount of myosin than skeletal muscle does, and not to an experimental error. Red muscle stands nearer to heart muscle inasmuch as it contains less extractable and therefore more stroma-N than white muscle.

We may mention that ALBURN and MYERS (1939) estimated the collagen content of different parts of dogs' hearts. They worked with the same method, of SPENCER, MORGULIS and WILDER (l. c.), which was used in our experiments. If we take the N-content of the dog's heart to be the same as that of cats' hearts (2.76%) we find, calculating with their data, that 15.5% of the total N-content is collagen-N. This value is in amazing accordance with our findings in cats' hearts according to which 15.7% of the total-N is Collagen-N.

In the second part of our experiments we wanted to find out whether the protein content or its distribution into different fractions shows any change during hypertrophy or whether there is in the course of the hypertrophy a phase in which a difference can be found. Therefore we carried out estimations at different times after the destruction of the aortic valves. The data here published are from hearts observed 8—10, that is 27—29 days after the valvular lesion. As there was not the slightest difference in the estimated values, the Tables of this paper contain the combined results. To decide the question finally, estimations should be carried out immediately (2—3 days) after the operation and several months later. The N-fractions of decompensated hearts should also be determined separately.

The conclusion to be drawn from these experiments is that — at least in the early and middle phases of the hypertrophy — the different N-fractions increase at the same rate, because their percentual proportions remained unchanged. We do not know what significance the fact of the N-content of hypertrophized heart-muscle being somewhat increased may have. This increase is about 7%. Since the N-content of the different fractions in normal and hypertrophized hearts

shows a great variability, this difference could hardly be valued even if the whole increase was due to the increase of a single N-fraction.

It was found in our earlier experiments on rats (l. c.) that the percentual dry matter content of the heart corresponds — even in the early phases of the hypertrophy — to the normal values. Table II. shows the same to be true in the case of cats' hearts.

Our experiments prove that as the quantitative and qualitative distribution of the heart's protein content remains unaltered, the heart increases its mass in such a way that the composition of the new matter corresponds exactly to that of the old.

SUMMARY.

1.) Cats' hearts contain somewhat less N than skeletal muscle does.

2.) A smaller part of the heart's N-content can be extracted according to Weber than of the skeletal muscle.

3.) Hypertrophized heart muscle contains somewhat more N than normal heart muscle.

4.) The distribution of the N-content into different fractions (myosin, globulin, myogen, Rest, collagen, remaining, fluid N) is the same in normal and hypertrophized heart muscle.

We wish to express our deepest gratitude to Professor A. B. L. Beznák for his constant help and advice during the course of this work.

Grateful acknowledgment is also made to Mrs. J. Thompson Vass for help in the English translation.

REFERENCES.

Alburn, H. E., Myers, V. C. (1939). *J. Biol. Chem.* **131.** 713.
Beznák, M., Hajdu, I. (1944). *Orvostud. Közl.* No. 14.
„ „ (1946). *Schweiz. Med. Wschr.* **76.** 390.
„ „ (1944). *Orvostud. Közl.* **35.** 700.
„ „ (1945). *Schweiz. Med. Wschr.* **75.** 300.
Bloor, W. R (1927). *J. Biol. Chem.* **72.** 327.
Bloor, W. R., Okey, G. W. R., Corner, (1930). *J. Biol. Chem.* **86.** 291.
Bloor, W. R. Cit. Bloor, W. R. Snider, R. H. (1934). *J. Biol. Chem.* **107.** 459.
Bloor, W. R., Snider, R. H. (1934). *J. Biol. Chem.* **107.** 459.
Detzel & Lang (1931). *Z. Physiol. Chem.* **201.** 157.
Deuticke, H. J. (1926). *Verh. d. ges. deutsch. Naturforsch. u. Aerzte. Sept. 19-26.* Ber. ü. ges. Phys. 38. 372.

Deuticke, H. J. (1930). *Pflügers Arch.* 224. l.
„ (1932). *Hoppe-Seylers Z.* 210. 97.
Hajdu, I. Beznák M. (1943). *Orvostud. Közl.* No. 19.
„ „ (1945). *Schweiz. Med. Wschr.* **75.** 665.
Hitchings, G. H., Wearn, J. T. (1941). *J. Biol. Chem.* **140.** LXI.
Kaufman, C. (1927). *Z. Geburtsh. u. Gynäk.* **91.** 668.
Ludewig St., Chanutin, A. (1936). *J. Biol. Chem.* **115.** 327.
„ „ (1936). *Arch. Int. Med.* **57.** 887.
Masuda, T., Bloor, W. R. (1932), *J. Clin. Inv.* **11.** 667.
Mayer and Schaeffer (1914). *J. Physiol. et Path. Gén.* **16.** 325.
Spencer, H. C., Morgulis, S., Wilder, V. M. (1937). *J. Biol. Chem.* **120.** 257.
Szent-Györgyi, A. et al. (1941). *Studies from the Inst. of Med. Chem. Univ. Szeged Vol. I.*
Weber, H. H., Meyer, K. (1933). *Biochem. Ztschr.* **266.** 137.

METHOD OF PRODUCING HEART HYPERTROPHY IN ALBINO RATS BY NARROWING THE AORTA ASCENDENS.*

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(With 1 Figure and 4 Tables in the text.)

(Received for publication 1st February 1944.)

To be able to work out the physiology and sphere of activity of the hypertrophized cardiac muscle we must have methods by which heart hypertrophy can be produced experimentally.

In our previous experiments (Hajdu and Beznák 1943; Beznák and Hajdu 1944) we produced heart hypertrophy by narrowing the aorta with a silver ring of a suitable diameter just below the diaphragm. (This stricture caused 30—50% hypertrophy in 5 days). From about the third day after the narrowing we found transsudation in the pleural cavity and possibly edema of the brain, which were not caused by heart failure. Only part of the deaths occurring were due to heart failure, the majority probably being caused by the mechanical effect of the great quantity of pleural fluid and by edema of the brain. We decided to seek another method, by which we could avoid the effects not caused by the changed haemodynamic conditions which made interpretation of the results more difficult. The new method consisted of narrowing the aorta just as it leaves the heart, before the branching of the great vessels. We hoped thus, by imitating the aortic stenosis more carefully, to avoid the disturbing factors above mentioned.

* The expenses of this work were met by a grant from the Natural Science Research Council, for which we take pleasure in expressing our gratitude.

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METHODS.

We worked on albino male rats, inbred for 4 years in our laboratory, weighing 185 ± 15 gs. The operation was performed in ether anaesthesia. After a median incision and stripping off the chest muscles we cut the sternum vertically at its joint with the IInd rib and, having freed it from the joint with the Ist rib and the clavicle, removed it. After the blunt separation of the deeper muscles and the thymus, the brachiocephalic artery came into sight. We prepared this artery down to the aortic arch. By slightly pulling the brachiocephalic artery we freed the ascending aorta so that we could get under it with a fine pair of crooked forceps. We put a silver ring (which was made previously and opened a little) under the aorta so prepared and closed it by mild pressure. After letting the aorta go we controlled the position of the ring and pushed it a bit down towards the heart. The wound was closed by skin suture. The operation can be performed after some practice without the opening of either pleural cavity; that is, without pneumothorax. Special care should be taken that the cutting of the sternum does not take place below the joint of the IInd rib (pneumothorax). The cutting of the Ist rib and the collar bone should be performed on a 45° inclined operating bank illuminated by a reflector. The rings were made of 0.8 mm wide silver wire. This wire was wound in tight spirals round a small steel cylinder possessing the required diameter. The spiral so prepared was sawed on one side along the axis and fell into as many rings as the wire was wound round the cylinder. We smoothed the edges of the rings and somewhat opened them. We tried rings with different diameters, using 10 animals for each diameter, to find the most suitable size. We found the best width to be 1.45 mm. The experiments here reported were all performed with this size. We made an electrocardiogram of the animals before the operation, just after it, and later once a week. The results thus obtained are published in the following paper. We dissected the animals immediately after their death, or after their being killed. First the atria and auricles of the hearts were cut off, opened, freed from blood and weighed. The right ventricle was cut off from the septum with a pair of fine scissors, dried and freed from blood. Then we cut off the left ventricle from the septum and weighed ventricle and septum separately. The measurements were performed on a torsion balance with an accuracy of ± 1 mg. We made determinations of dry weight and ash content in the right part of the heart (right ventricle, septum and atria) and in the left part (only the wall of the left ventricle.).

EXPERIMENTAL RESULTS.

We narrowed the aortae of 95 rats in the above described way. The mortality curve of 82 rats is shown in Figure 1. Eight fur-

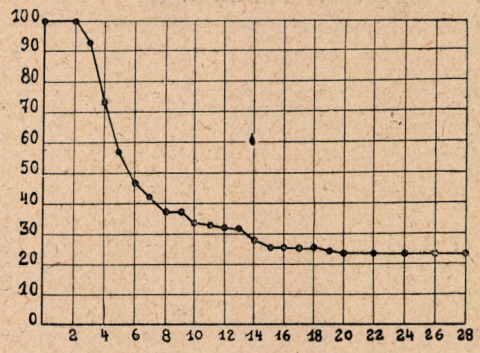

Figure 1. Survival of rats after the narrowing of their aorta ascendens. X-axis: days after the operation. Y-axis: survival of rats in %.

ther rats were killed on the second day and 5 died during the operation (3 from pneumothorax, 2 from bleeding). It is seen from this Figure that the mortality is very high between the 3rd and 7th days. During that time 58% of all the animals die. During the 2nd and 3rd week after the narrowing, mortality becomes increasingly rare, so that during that time only 19% of the animals die. There is no mortality from the 3rd week to the end of our experimental period (28 days). Dissection of the 48 animals which died during the first week after the operation showed haemothorax or haemopericardium in 46, the cause of these being the rupture of the aorta just below the ring. In the animals which died during the 2nd and 3rd weeks we found the following symptoms: dilated heart filled with blood, plethoric, brownish lungs which were darker than normal and from which frothy liquid could be pressed out, a small amount of fluid in the thoracic cavity, swollen liver with well defined structure. These findings showed that the cause of death was cardiac failure. In the 18 rats which survived to the 28th day we found a small edema of the somewhat pigmented lungs and explicit hyertrophy of the heart; that is, findings corresponding to a state of compensated vitium. Very often we observed fusiform aneurysmata of the aortae below the stricture, the size of which varied from that of a coloured pinhead to that of a pea. The death statistics are therefore the following: of the 82 animals about 60% died in consequence of rupture of the aorta, 20% in consequence of decompensation, and 20 remained living in

a compensated state. As was stated, we killed 8 animals on the second day to decide the onset of hypertrophy in relation to time.

The weights of the hearts and their parts are given in Table I.

TABLE I.

Changes in wet weight of certain parts of the heart.

	No.	Weight of whole heart		Change in % compared with normal	Sign. diff.	Weight of left ventr.		Change in % compared with normal	Sign. diff.	Weight of right ventr.		Change in % compared with normal	Sign. diff.	Weight of atria		Change in % compared with normal	Sign. diff.
		mg.	± μ			mg.	± μ			mg.	± μ			mg.	± μ		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Normal	31	631	59	—	—	405	38	—	—	162	27	—	—	64	13	—	—
2 days after narrowing	13	717	106	+ 14	2.8	477	81	+ 18	3.1	160	27	− 1	0.2	80	17	+ 25	3.0
Aortic rupture (first week)	43	721	73	+ 14	5.9	479	87	+ 18	5.0	168	27	+ 4	0.9	74	16	+ 16	3.0
28 days after narrowing	18	812	161	+ 29	4.6	547	116	+ 35	5.0	172	36	+ 6	1.0	92	20	+ 44	5.4
Decompensation	16	83›	161	+ 32	4.9	530	99	+ 31	4.9	203	49	+ 25	3.1	102	31	+ 59	4.6

We divided the weight of the septum in proportion to the weight of the two ventricles and added that weight to the one measured. In this way we got the weights of the right and left ventricles. These weights are given in the Table. It is seen from columns 2 and 4 of this Table that the hearts show a hypertrophy of 14% on the 2nd day after the narrowing. This hypertrophy hardly increases till the end of the first week but is almost doubled by the end of the experimental period (28 days). Columns 6—17 show that the left ventricles and the atria are hypertrophized. Their hypertrophy, like that of the whole heart, hardly changes from the 2nd to the 7th day and is doubled by the 28th day. It is seen in columns 2—7 that the right ventricle does not take part in these changes.

Table II. shows what part the different portions of the heart play in the hypertrophy found at different times. It is seen that the left ventricles make up about 20% of the whole hypertrophy, and 20% is produced by the atria.

We discuss separately the findings of those animals where the cause of death was decompensation. These results are shown in the last line of Tables I. and II. In these animals the hypertrophy was

TABLE II.

The distribution of hypertrophy in %.			
	Left ventricle	Right ventricle	Atria
2 days after narrowing	81.4	0.0	18.6
7 days after narrowing	82.2	6.7	11.1
28 days after narrowing	78.9	5.5	15.6
Decompensation	61.3	20.1	18.6

rather great: 32%. It is seen that here not only the left ventricles (Table I. columns 6—9) and the atria (columns 14—17), but also the right ventricles show hypertrophy (columns 10—13). Of the 32% hypertrophy 60% is produced by the left ventricles, and 20—20% by the right ventricles and the atria. (Last line of Table II.)

The results in dry weight are shown in Table III. In this and the following Table "left side" means only the wall of the left

TABLE III.

Dry matter content in %.							
	1. No.	2. Left %	3. side ±	4. Signif. diff.	5. Right %	6. side ±	7. Signif. diff.
Normal	18	22.0	1.35		22.2	1.49	
2 days after narrowing	8	20.8	1.10	2.3	18.5	2.20	4.2
„Narrowed group"	28	21.9	0.75	0.3	20.8	1.20	3.1
Decompensation	7	21.8	1.14	0.4	21.1	1.88	1.3

ventricle as cut off from the septum, and "right side" means the wall of the right ventricle + the septum + the atria. The "narrowed group" contains all the animals which did not decompensate. We took as a separate group only those that we killed on the 2nd day after narrowing. It is seen from this Table that on the left side there is no appreciable difference in the content in dry material; that means that wet and dry materials take part in the resulting hypertrophy to about the same extent. The gain in wet weight somewhat over-rides that of dry matter on the 2nd day. (Table III. 2nd line, columns 2 and 4.) In spite of there being no change in the weight of the right side (right ventricle+septum+atria) on the 2nd day, there is a significant decrease in dry matter. (Table III, line 2, columns 5—7). Later on, when the weight of the right side increased (as we measured the hypertrophic septum and atria with the right side), the decrease in dry matter is less. In decompensated animals the right ventricle also hypertrophizes

(Table I, columns 12 and 13, last line) and then there is no change in dry matter. (Table III., columns 5—7, last line).

Table IV. shows the ash content in pro mille. It is seen that the ash content increases only on the 2nd day of the narrowing; later on it returns to normal. (Table IV, columns 4 and 7).

TABLE IV.

	Ash content in pro mille.						
	1. No.	2. Left %	3. side ±	4. Signif. diff.	5. Right %	6. side ∓	7. Signif. diff.
„Narrowed group"	18	10.6	0.80	—	10.0	1.40	—
2 days after narrowing	8	11.3	0.60	2.2	10.2	0.90	0.3
Normal	28	10.7	0.94	0.4	9.5	1.00	1.3
Decompensation	7	10.8	0.51	0.8	10.3	1.10	0.5

DISCUSSION.

In these experiments, we tried to work out a method by which we could imitate conditions of human aortic stenosis. The difference between our method and pathological conditions is only that in our case the coronary arteries originate below the stricture; that is, between the stricture and the heart; whereas in pathological conditions they originate after the stricture. With this method we could avoid those disturbing influences seen after the narrowing of the abdominal aorta (edema of the brain, great transsudation in the pleural cavity), and the deaths of those animals that were not killed by rupture of the aorta we knew to be due to heart failure. Our method is therefore well suited for producing heart hypertrophy in rats and for investigating the physiology of compensated and decompensated hearts.

Experimental aortic stenosis, just as that seen in pathological conditions, imposes work chiefly on the left ventricle. Thus the left ventricle shows a hypertrophy from the 2nd day on, so that it is responsible for 80% of the hypertrophy. The wet weight of the right ventricle, which at this stage does not perform plus work, does not change. The atria hypertrophize like the ventricles from the 2nd day on (15—20% of the hypertrophy). Under the influence of long-lasting stricture the hypertrophy may reach a very high value (44% of its original weight). In the animals that died in consequence of decompensation and where the circulation in the lungs was also hampered, there

was a marked hypertrophy of the right ventricle. In these cases the hypertrophy of the atria was even more marked (They increased by 60% of their original weight).

The dry weight in percentage and the ash content pro mille of the left ventricle do not change appreciably during the whole of its hypertrophy. The increase in fluid is a bit more than that of dry matter in the hypertrophy produced at the end of the 2nd day. (Table III., column 4. Sign. Diff.: 2.3). The right ventricle behaves somewhat differently. Though its weight does not change there is a significant diminution of dry elements. As at the same time the ash content $^0/_{00}$ is unchanged, the only possible explanation is that the right ventricle lost organic materials which were replaced by some fluid having the same ash-content as the heart. The greater the wet weight of the right ventricle becomes, the smaller the loss in organic compounds. In the case of decompensation where the right ventricle also hypertrophizes, this loss is altogether absent: in that case the dry matter in % has the same value as in normal hearts.

As was mentioned, in the beginning hypertrophies (2nd day) when the hypertrophy is caused by retention of water, the inorganic materials of the heart increase; this increase is only temporary, for in the later, real, phase of the hypertrophy it is again absent. This observation is in good agreement with that of Hitchings and Wearn (1941), who found that the electrolyte content of rabbits' hearts increases in the 3 first days of the hypertrophy but regains its normal level by the 6th day.

SUMMARY

1.) We publish a new method by which we can produce hypertrophy of the heart by artificial narrowing of the ascending aorta.

2.) 60% of the animals treated in that way (95 cases altogether) die within the first week in aortic rupture; 20% die in the course of the 2nd week in circulatory decompensation; 20% live on in a compensated state.

3.) Out of the total hypertrophy thus developed, 80% is that of the left ventricle, 20% that of the atria. The right ventricle hypertrophizes only in decompensation.

4.) The dry weight (in %) and the ash content (pro $]_0{}'^0$ increases during the first days of the hypertrophy; later on they both regain the normal level.

We have pleasure in expressing our deepest gratitude to Professor A. B. L. Beznák for his constant help and advice during the course of this work.

Grateful acknowledgment is also made to Mrs. J. Thompson Vass for help in the English translation.

REFERENCES.

Beznák M., Hajdu I.: (1944). *Orvostud. Közl.* **14.** 326.
1946. *Schweiz. Med. Wschr.* **76.** 390.
Hajdu I., Beznák M.: (1943). *Orvostud. Közl.* **19.** 571.
1945. *Schweiz. Med. Wschr.* **75.** 665.
Hitchings G. H., Wearn J. T.: (1941). *J. Biol. Chem.* **140.** LXI.

CHANGES IN THE ELECTROCARDIOGRAM PRODUCED BY THE NARROWING OF THE ASCENDING AORTA IN WHITE RATS.

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(With 3 Tables in the text.)
(Received for publication 1st June 1944.)

In our preceding communication (Hajdu and Beznák, 1946) we published a method by which, through the narrowing of the ascending aorta, a state was produced in many respects similar to the aortic stenosis known in human pathology. To get a picture of the condition of the heart an electrocardiogram was made at different times after the narrowing. Our present communication contains the results and evaluation of these observations.

METHODS.

The electrocardiograms were made on the experimental animals of the preceding communication. The rats lay on their stomachs in light ether anaesthesia. Needles were pushed through the skin in the usual Einthoven leads and records were taken with a Swedish Elmquist electrocardiograph. The first electrocardiogram was made immediately before, the second immediately after the narrowing. Afterwards a record was taken each week until the deaths of the animals. Our results were based on the electrocardiograms of 40 rats. In one case, only the record before, and in one, only that after the narrowing was available. There were 2 records after the narrowing in 19 cases, 3 records in 11 cases, 4 records in 11 cases and 5 records in 11 cases.

EXPERIMENTAL RESULTS.

Table I. shows the cardiac frequency of rats before, immediately after the narrowing, and later once every week. It is seen from the Table that the cardiac frequency of normal rats is 436±58/minute. This decreases to 329±43/minute immediately after the narrowing. This decrease is transitory, as the frequency returns to normal 1 week after the narrowing and decreases only during the 4th week. The decrease amounts to —24% immediately after and to —18% four weeks after the narrowing.

TABLE I.

Cardiac frequency/min. of rats																	
Normal			Immediately after the narrowing			1 week			2 weeks			3 weeks			4 weeks		
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
38	436	58	37	329	43	19	420	47	11	448	42	11	420	34	11	357	84

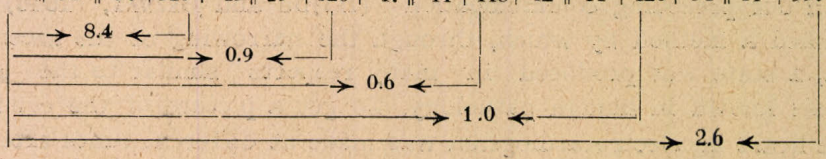
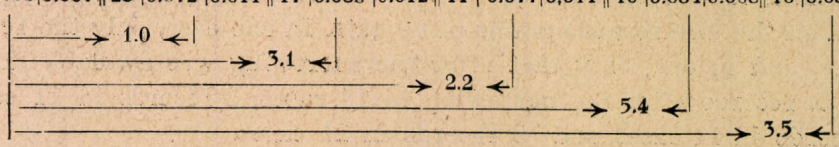


Table II shows the length of the electric systole (Q—T distance) in σ. In normal animals this is 70±7σ; it gets gradually longer in consequence of the narrowing. Immediately after the narrowing it has the same value as normally, it is 14% longer on the first, 10% longer on

TABLE II.

Duration of electric systole (Q—T distance) in seconds																	
Normal			Immediately after the narrowing			1 week			2 weeks			3 weeks			4 weeks		
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
34	0.070	0.007	25	0.072	0.011	17	0.080	0.012	11	0.077	0,011	10	0.084	0.008	10	0.086	0.014



the second, 20% longer on the third, and 23% longer on the fourth week.

Table III. is constructed of the data of Tables I. and II. It shows what percentage the electric systole makes of a whole revolution. It is seen from this Table that normally an electric systole takes up 50% of a whole revolution. As the heart rate slows down immediately after

TABLE III.

An electric systole as % of a whole revolution

Normal			Immediately after the narrowing			1 week			2 weeks			3 weeks			4 weeks		
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
34	50	4	25	39	7	17	55	7	10	56	8	10	59	9	10	48	10

→ 6.6 ←
→ 2.7 ←
→ 2.3 ←
→ 3.0 ←
→ 0.6 ←

the narrowing and the duration of the electric systole is unchanged, it occupies a smaller part — only 39% — of a whole revolution. Later the electric systole becomes longer (Table II.) and the frequency increases to the normal value (Table III.); correspondingly an electric systole takes up a greater part of a whole revolution during the 1st, 2nd and 3rd weeks. The situation changes during the 4th week, when the electric systole is longer than normal, but the heart rate is again — if to a smaller extent — slower. At this period the electric systole consists of 48% of a whole revolution, a value statistically equivalent to the normal 50%.

The atrio-ventricular conduction time (P—Q distance) is $47 \pm 4\sigma$, in normal rats. Immediately after the narrowing its value increases to $51 \pm 5\sigma$, but percentually takes up a smaller part of a whole revolution (28% instead of the 33% normal value); that is, it behaves similarly to the electric systole. Later on the atrio-ventricular conduction time shows no change.

The T-waves are positive in normal rat electrocardiograms, here and there a small ST depression occurs in the 1st lead.

In one rat we observed ventricular premature contraction and in two, transitory a-v block in normal condition. The rat which normally showed ventricular extrasystoles lived 28 days after the narrowing in clinically compensated condition. Of the two animals showing a-v block

normally, one lived also for 28 days in compensated condition; no signs of block were seen on later records. The other rat died in consequence of the rupture of the aorta before we were able to take a second electrocardiogram. On the record taken immediately after the narrowing no a-v block was seen.

Qualitatively, the following changes in the electrocardiogram may be observed after narrowing the ascending aorta: The T-waves become flat immediately after the narrowing, later they become transitorily positive again. In the animals living longer than 2 weeks after the narrowing, the T-waves again become flattened and remain thus until the end of the experimental period (28 days).

A longer or shorter time after the narrowing ST depression occurred in 13 rats in one, two or all the three leads. We observed auricular extrasystoles in two, ventricular extrasystoles in two, auricular fibrillation in two, sinoauricular block in one, partial block (3:1, 2:1) in two and total atrio-ventricular block in one case.

Comparing the changes of the electrocardiograms with the clinical observations of the rats and their autopsy findings, the following statements can be made:

There is no correlation between the initial heart rate, the ensuing bradycardia and the clinical state of the animals. As we stated in our preceding paper, 60% of the rats died in consequence of aortic rupture during the first 10 days; 20% of the animals showed signs of decompensation, and 20% remained alive until the end of our experimental period (28 days).

Changes in the electrocardiograms appeared at least one week, generally two weeks after the narrowing. Accordingly there is but little change to be seen on the electrocardiograms of the rats dying in the course of the first 10 days as a consequence of aortic rupture. Only in 5 of 21 animals which died thus did we observe a flattening of the T-waves in all the three, and in one in the 1st lead only. In three of these ST depression also occurred in the 1st lead during the course of the 1st week. In one animal of the above 5, a partial block (2:1 resp. 3:1) also occurred.

The other changes described — as they generally appear only after the second week — were seen in rats living longer than this period; that is, in rats dying either in decompensation or living in a compensated state for 28 days. There was no correlation between the severity of the changes in the electrocardiograms and the state of the animals; i. e., compensation or decompensation.

In five cases we observed a marked rise of the P-wave which at

the same time became more pointed (P-pulmonale) in the IInd and IIIrd lead. Three of these were seen in decompensated animals. In one case P-pulmonale was observed already one week after the narrowing, in one on the 2nd, in one on the 4th week. In two cases it was seen in rats which lived for 28 days in a compensated state and where on autopsy we found a relatively small heart and "induratio brunea" of the lungs. Of these one developed during the 2nd, and one during the 3rd week after the narrowing.

DISCUSSION.

By narrowing the ascending aorta we produce a state in many ways resembling the aortic stenosis seen in human beings. From the electrocardiograms taken immediately after the narrowing a sharp fall in the heart rate can be observed. Similar results were gained by DALY and VERNEY (1927), who increased the pressure in the left ventricle and found bradycardia if the vagi were intact. This bradycardia is, however, only temporary, for 1 week after the narrowing the frequency is again normal. The bradycardia of the 4th week is different from that seen immediately after the narrowing. It is observed in every animal immediately after the narrowing, whereas in the 4th week a sharp decrease in frequency takes place in some animals, while others scarcely change. That accounts for the greater deviation of the mean value.

The electric systole becomes longer after the narrowing. Immediately after the narrowing its value is the same as normally but as the heart rate is slowed down at the same time, the electric systole takes up a smaller part of a whole revolution than it normally does. This means that in the ensuing bradycardia only the diastole becomes longer. The conduction is unchanged but the impulse formation becomes slower. Later the time of the electric systole is longer. The frequency being normal at the same time, the electric systole takes up a greater part of a whole revolution than normally. During the 4th week bradycardia is again present, with longer electric systole at the same time, so that the relation of electric systole to whole revolution is the same as under normal conditions. Somewhat similar results were obtained by RASSMUSSEN (1942), who found that the QRS complex changed in dogs from 0.03—0.04 sec. to 0.07—0.09 sec. after the ligature of the aorta. The changes generally observed after the 2nd week are: flattening of the T-waves, St depression, in some cases block. These symptoms occur with the same frequency in decompensated rats and in those living 28 days

in a compensated state. In these experimental conditions, therefore, no conclusion can be drawn from the changes in the electrocardiograms of the clinical state of the rats.

It is interesting to note that the same experimental procedure — aortic stenosis — produces very varied changes in the impulse formation and conduction. We cannot say that a definite procedure — narrowing the ascending aorta — produces such and such a change in the electrocardiogram. Behind each change the breaking down or disturbance of a definite biochemical process is to be suspected. Depending on the individual hearts, the same experimental procedure produces a disturbance in varied biochemical processes, the results of which are the manifold changes in the electrocardiograms.

SUMMARY.

Electrocardiograms taken of 40 rats before, immediately after, and then weekly after narrowing the ascending aorta show the following changes:

1.) Narrowing the ascending aorta causes bradycardia. This is transitory, for the heart rate becomes normal one week later and a decrease occurs again only 4 weeks later.

2.) A later consequence of the narrowing is the lengthening of the electric systole.

3.) The T-waves flatten immediately after the narrowing. Later they may again become transitorily positive.

4.) In the majority of cases ST depression occurs.

5.) Observed changes are: auricular extrasystoles (2 cases), ventricular extrasystoles (2 cases), auricular fibrillation (2 cases), sino-auricular fibrillation (2 cases) sino-auricular block (1 case), partial block (2 cases), total auriculo-ventricular block (1 case).

6.) P-pulmonale was observed in 5 cases.

Grateful acknowledgment is made to Mrs. J. Thompson Vass for help in English translation.

LITERATURE.

Beznák M., I. Hajdu: *Arch. Biol. Hung.* This. number.
Daly I. de Burgh, E. B. Verney: (1927). *J. of Physiol.* **62.** 330.
Rassmussen H.: (1942). *Acta med. scand. (Stockholm)* **110.** 32.

THE AUTONOMIC INNERVATION OF THE HEART IN REGULATING THE BLOOD SUGAR.

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With 3 figures in the text.

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It is known from the work of several authors that the ligature of an artery increases the blood sugar level. This was found by ligature of the carotid artery in rabbits (Thelen, 1933) and in dogs (Brauch, Euler and Liljestrand, 1934). Because of some discrepancy in their results, Hungerland (1936) repeated the experiment. He found that ligature of both carotid or both femoral arteries caused an elevation of the blood sugar in unanaesthetised dogs, but that it failed to do so if the dog was anaesthetized with morphine-Pernocton. Zimmermann-Meizinger (1935) described a slight hyperglycaemia and glycosuria during the first hours following myocardial infarction. Cesare and Bellini (1936) proved that in guinea pigs simple heart puncture produced hyperglycaemia, even in animals anaesthetized with Dial, where the psychic effect was excluded. After F 933 administration no blood sugar elevation took place. Since the ligatured arteries supply organs playing a very different rôle in the blood sugar regulation (leg muscles: femoral artery, brain: carotid artery, heart: coronary artery) and since these authors recorded their observations without having made experiments in order to elucidate the physiological mechanism of the phenomenon observed, the whole question is in want of a more detailed experimental study.

We investigated the blood sugar curves of cats before and after the ligature of the anterior descending branch of the coronary artery. We wanted our experiments to elucidate the following questions:

1.) Does ligature of the coronary artery increase the blood sugar

level? If it does, how great and how lasting is the hyperglycaemia thus produced?

2.) By what mechanism does coronary artery occlusion produce hyperglycaemia? Is it a question of some hyperglycaemia-producing substance getting into the blood from the heart infarct, or is it produced through reflex mechanism?

As it is known that anaesthesia, thus ether anaesthesia too (Mekie and Miller, 1929; De Fermo, 1930; Fuss, 1930; Ferradas, 1932; Eisler and Hemprich, 1932; Reid, 1932; Müller, 1932), as well as surgical shock (Amorosi, 1932; Guer reri, 1931; Guiseppe, 1933; Weddel and Gale, 1934; Banaitis, 1936; Stolfi, 1936) produce hyperglycaemia, we excluded the effect of these influences by control experiments.

METHODS.

Our experiments were performed on cats of both sexes weighing 2.7 ± 0.6 kg, which had fasted for 24 hours. The animals, lying on an electrically heated operating table, were observed in ether anaesthesia with artificial respiration for 8—9 hours. Blood pressure was measured in the left femoral artery by Trendelenburg's method* and blood samples for sugar analysis were drawn from a side branch of the right femoral artery. Normal blood sugar level was determined 3 times with an interval of 20 minutes. The chest and pericardium were opened and the anterior descending branch of the coronary artery was ligatured, taking care not to injure anything during the preparation of the artery; the chest was then closed. 40, 80 and 120 minutes after the ligature, then every hour, the blood sugar content of the arterial blood was determined. In a second series of experiments, after the opening of the chest the stellate ganglia were prepared, from the chest, its branches running towards the mediastinum and the branches of the vagi running to the heart were cut, leaving the vagi themselves intact; and the chest was closed. In a third series, having performed the operation just described, we waited till the blood pressure stabilized, then re-opened the chest, then ligatured the anterior descending branch of the coronary artery. In the control series all operations were performed without either cutting the nerves or ligaturing the artery. In some experiments the rectal temperature of the cat was also registered simultaneous to the blood sugar determinations. Blood sugar analyses were made with the

* The authors are glad to thank the La Roche Firm (Basel) for a gift of „Liquoid"

modified HAGEDORN JENSEN method (FUJITA-IWATAKI, 1931). The state of the heart and the functional effect of the coronary occlusion was determined by taking electrocardiograms. Two records were taken from every animal, one before, and one 2—3 hours after the ligature of the coronary artery. Results of these records will be published in another paper.

EXPERIMENTAL RESULTS.

Our work was done on 50 cats. Results of 46 could be used, 4 dying in consequence of different causes, not depending on the experi-

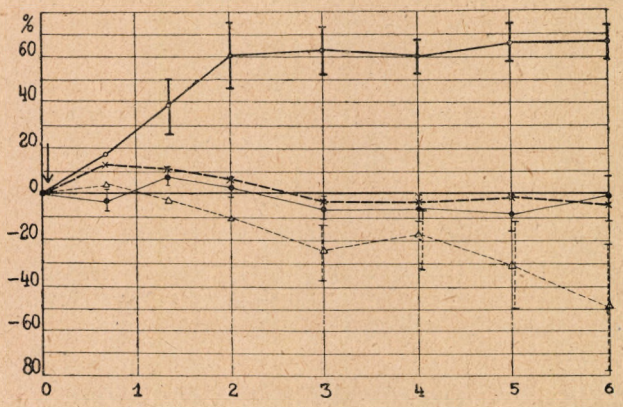

Figure 1. Changes in blood sugar level in %. X-axis: time after operation in hours. Y-axis: blood sugar changes in %. The time of the operation is shown by the arrow. ●——● : normal, ○——○ : coronary ligature, ×– – –× : nerve cutting + coronary ligature, △– – – –△ nerve cutting.

mental procedure. Figure 1. shows the percentual changes of the blood sugar curves, the normal curve being constructed from results on 7, the coronary ligatured on 8, the denervated on 8, and the denervated plus ligatured curve from results on 9 animals. The vertical lines at the different points indicate the standard deviation of the mean of the blood sugar determinations carried out at that point. For the sake of simplicity the deviation is not shown at those points where values almost cover each other. The time of the operation (ligature of the coronary artery, denervation plus ligature, control operation) is shown by the arrow. It is seen from this Figure that ligature of the anterior descending branch of the coronary artery causes an elevation of blood sugar, beginning at the 40th minute and attaining its maximum (60—65%) at the 120th minute, and remaining at the

same level even 6 hours after the ligature. Coronary occlusion causes no alteration of the blood sugar level if the autonomic nerves of the heart had previously been cut. Cutting the nerves in itself lowers the blood sugar level, beginning at the 2nd hour and becoming more pronounced as time proceeds.

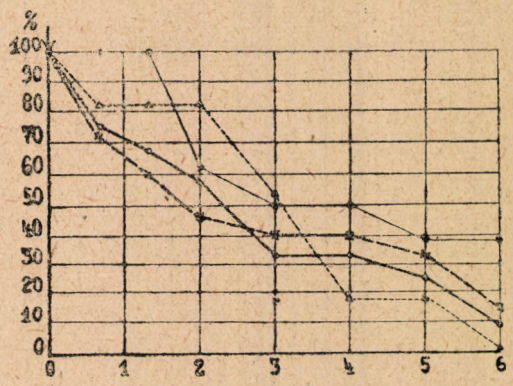

Figure 2. Changes in blood pressure in %. X-axis: time after the operation in hours. Y-axis: blood pressure changes in %. The time of the operation is shown by the arrow. ●——●: normal, ○——○ coronary ligature, ×– – –× nerve cutting + coronary ligature, Δ– – – Δ nerve cutting.

Figure 2. shows percentual changes of the mean arterial blood pressure of the same groups. The time of the operation is shown by the arrow. It is seen from this Figure that blood pressure falls immediately after the operation by about 30—50%. This proves that the fall is caused — at least chiefly — by the base operations (opening of the chest, pericardium, etc.).

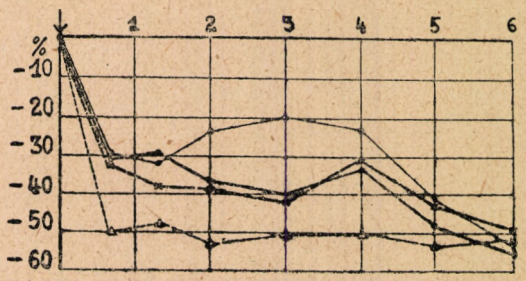

Figure 3. Survival of cats. X-axis: time after the operation in hours. Y-axis: survival of cats in %. ●——● : normal, ○——○ coronary ligature, ×– – –× : nerve cutting + coronary ligature, Δ· · · · · Δ nerve cutting

Figure 3. shows survival of the different groups in %. The normal curve contains 8, the curve of coronary ligature 12, of denervation 11, and of denervation plus ligature 15 animals. This Figure shows that initial mortality is absent in the normal group, the animals beginning to die only after the 2nd hour. 6 hours after the operation 40 % of the animals still live, whereas from the three experimental groups at the same time only 0—15% is surviving. The initial mortality is the greatest in those groups where the coronary artery has been ligatured (irrespective of denervation); it is smaller in the group where only denervation of the heart took place. There is no significant difference in the late mortality of all three experimental groups.

Lastly we may mention that the rectal temperature of the cat falls by 1—1.5° C after the opening of the chest, then gradually increases and either reaches the initial value or remains slightly below it.

DISCUSSION.

Our experiments definitely prove that ligature of the coronary artery produces without exception hyperglycaemia, and that this hyperglycaemia exists for hours. Our results differ in this respect from those of HUNGERLAND (l. c.) who found that the hyperglycaemia in consequence of the ligature of both carotid or both femoral arteries in the unanaesthetized dog is more transient, having passed in 2 hours. This difference is probably due to the circumstance that the ligature of a coronary branch causes an infarct in a vitally important organ, whereas ligature of the femoral or carotid artery merely reduces temporarily the blood flow of a more or less important region.

The question as to whether hyperglycaemia was produced by some accumulated metabolites in the thrombotic region getting into the blood stream, or by a reflex mechanism, was decided in our experiments in favour of the second hypothesis. Our experiments decide at the same time how the impulses travel from the heart to the central nervous system. In the group where denervation preceded the ligature of the coronary artery, detailed results were as follow: In 6 cases out of 9 the blood sugar showed no alteration; in 2 it increased — this increase being much smaller and in no way comparable to that obtained after ligature of the coronary artery in normal hearts; and in 1 case it fell to such an extent that it may be said to have disappeared altogether. The united curve constructed from all 9 data shows no change as compared to the normal curve (see Fig. 1.). From these experiments the

conclusion is warranted that the impulse travels to the central nervous system — at least in major part — via the autonomic nerves.

Special attention is drawn to the fact that denervation of the heart in itself influences the blood sugar level. In 4 out of 9 cases there was a very marked fall from the beginning, in 2 the blood sugar level hardly changed, and in 3 there was an initial increase. In 2 of these latter three the rise was slight; in 3 hours the blood sugar value returned to the normal level, then decreased. In 1 case the rise was very great. The curve of this group, seen in Figure 1. is constructed without this last animal, the type of blood sugar changes of the other 8 animals being similar: the blood sugar hardly changes or shows a very slight rise during the first hours and decreases during the last hours. Very different from this type is the one animal mentioned above. This rise of blood sugar was the greatest of all animals observed: 186% in the 3rd, and 175% in the 4th hour; after this the animal died. We do not know the cause of the special behaviour of this cat. It is possible that the operation caused greater destruction — through there is no sign of it in the experimental records; it is possible that this cat was more sensitive towards surgical operations and the blood sugar rise came from areas that were not denervated; and lastly it is possible — though very unlikely, no change exceeding 15% ever being observed in normal animals — that it was caused by the anaesthesia. We think that the observation that denervation of the heart causes — with one exception — a fall in blood sugar, may prove to be significant. Our experiments prove that the blood-sugar-regulating mechanism is unable to function normally if impulses coming from the heart region through autonomic afferent nerves are excluded by cutting these nerves. Clearly the heart plays a rôle in regulating the normal blood sugar level. According to our experiments, stimuli arise continuously in the heart that help in maintaining normal blood sugar level. If we cut the nerves of the heart, these impulses not being able to reach the central nervous system, a fall in the blood sugar level takes place. If, on the contrary, more impulses originate in the heart (as after coronary ligature) and if they are able to reach the central nervous system through nervous channels, the blood sugar increases.

We made no further experiments to decide how the impulses, having once reached the central nervous system, bring about the hyperglycaemia. It is difficult to explain it through simple adrenaline secretion, for blood pressure and blood sugar behave independently. The

blood pressure shows a sharp fall after coronary occlusion, denervation, and denervation plus coronary occlusion, yet at the same time very different changes in the blood sugar level occur.

Lastly, we have to mention briefly the fact — contrary to the data of the literature — that in our experiments we found no change in the blood sugar level in the course of long (approximately 8 hours) ether anaesthesia (Fig. 1.). We think that the cause of this discrepancy is that anaesthesia in our case was very superficial and, in consequence of the artificial respiration, very even throughout the whole experimental period.

SUMMARY.

1.) Ligature of the anterior descending branch of the coronary artery causes, in cats anaesthetized with ether and in artifical respiration, a well pronounced hyperglycaemia. The rise of the blood sugar begins round the 40th minute, attains its maximum in the 2nd hour and remains on that level even 6 hours after the ligature.

2.) The hyperglycaemia caused by coronary occlusion is absent.

3.) The cutting of the branches of the stellate ganglia and of the vagi leading to the heart produces in itself a fall in the blood sugar level, beginning some hours (approximately 3) after the cutting.

4.) There is no correlation between blood pressure and blood sugar level under these experimental conditions.

5.) Ether anaesthesia — very superficial and even — has no effect on blood sugar.

6.) The experimental results are discussed and a hypothesis, concerning the rôle of the heart as a receptor region in influencing the blood sugar level, is advanced.

We wish to express our gratitude to Professor A. B. L. Beznák for his constant help and advice during the course of this work.

Grateful acknowledgment is also made to Mrs J. Thompson Vass for help in the English translation.

LITERATURE.

AMOROSI O. (1932). *Arch. ital. Chir.* **29.** 521.
BANAITIS S. J. (1936). *Trudy voemo-med. Akad. Kiroa.* **5.** 111.
BRAUCH F. (1934). *Naunyn-Schmiedebergs Arch.* **175.** 104.
CESARE A., BELLINI L. (1936). *Biochemicae Ter. sper.* **23.** 401. 101.
DE FERMO CESARE. (1930). *Arch. ital. Chir.* **25.** 356.
EISLER B., HEMPRICH R. (1932). *Z. exper. Med.* **83.** 439.
EULER N. S. v., LILJESTRAND G. v. (1934). *Skand. Arch. f. Physiol.* **71.** 73.
FERRADAS J. (1932). *Rev. sud-Amer. Med. (Paris)* **3.** 39.
FUSS H. (1939). *J. exper. Med.* **73.** 506.
FUJITA-IWATAKI (1931). *Bioch. Z.* **242.** 43.
GUERRERI G. (1931). *Ateneo parm.* II. 5. 3. suppl. 184.
GUISEPPE L. (1933). *Ann. ital. Chir.* **12.** 137.
HUNGERLAND H. (1936). *Naunyn-Schmiedebergs Arch.* **181.** 435.
MEKIE E., MILLER H. (1929). *Brit. med. J. Nr.* **3553.** 244.
MÜLLER H. (1932). *Arch. Ohr. usw. Heilk.* **133.** 169.
REID CH. (1932). *J. of Physiol.* **75.** 40.
STOLFI G (1936). *Arch. di Sci. biol.* **22.** 583.
THELEN P. (1933). *Z. f. d. ges. exper. Med.* **86.** 231.
WEDDEL A. G GALE H. E. D. (1934). *Brit. J. Surg.* **22.** 80.
ZIMMERMANN-MEINZINGER O. (1935). *Z. klin. Med.* **129.** 269.

EXPERIMENTELLER VERLETZUNGSSCHOCK BEI WEISSEN RATTEN.*

Von: TIHAMÉR CSÁKY und GYULA MAGYARY-KOSSA.

Aus dem Physiologischen Institut der Péter-Pázmány-Universität Budapest und dem Ungarischen Biologischen Forschungsinstitut Tihany am Balaton.

Mit 3 Abbildungen und 2 Tabellen im Text.

(Eingegangen am 4. März 1944)

Unter sekundärem Verletzungsschock versteht man den gewisse Zeit nach erlittenem Trauma, zufolge umfangreicher Gewebsläsion entstandenen schweren, mit niedrigem Blutdruck einhergehenden, oft tödlich ausgehenden Zustand. Hinsichtlich dessen Entstehungsmechanismus liessen sich viele Fragen aus dem Grunde nicht bereinigen, weil zwecks Anstellung einer entsprechenden Versuchsserie eine grosse Anzahl von Versuchstiergruppen erforderlich wäre. Von Laboratoriumstierarten ist die weisse Ratte hierfür am geeignetsten, weil sie leicht züchtbar ist; demzufolge gelingt es unschwer, aus so vielen Tieren bestehende Gruppen zu gestalten, deren Mitglieder gleichartig sind, aus der gleichen Zucht stammen und unter identischen Lebensbedingungen existieren. Dagegen ist es bisher nicht gelungen, bei Ratten einen Schock hervorzubringen.

Unsere unten zu besprechenden Versuche haben folgendes zum Gegenstande: 1. Wir bemühten uns um die Ausarbeitung einer Methode für das Hervorrufen eines experimentellen Verletzungsschocks auch bei der weissen Ratte, 2. Wir untersuchten, ob bei der Entwicklung des traumatischen Schocks der weissen Ratte ein humoraler Faktor eine Rolle spiele.

Als erste versuchten V o e g t l i n und D y e r (1927) bei der Ratte den experimentellen Verletzungsschock durch kräftige mechanische Lä-

* Die Arbeit wurde im Jahre 1943 fertiggestellt und „Naunyn Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie" eingesandt, doch konnte sie wegen der Kriegsverhältnisse nicht veröffentlicht werden.

sion der Tiergliedmassen hervorzubringen; der Versuch blieb aber erfolglos, indem die Tiere auf diese Weise einen Schock nicht bekamen. Die gleichen Verfasser fanden auch eine aussergewöhnliche Resistenz der weissen Ratte gegenüber Histamin. Andere Tierarten erwiesen sich Schock gegenüber als wesentlich empfindlicher, und dem parallel war auch ihre Histaminempfindlichkeit grösser. So wurde denn auch die Beobachtung Voegtlin u. Dyers ein Beweis für die Auffassung, dass Ursache des sekundären traumatischen Schocks das Freiwerden von Histamin oder eines histaminartigen Stoffes sei. Diese Auffasung scheint von einem anderen Umstande noch mehr unterstüzt zu werden. Exstirpiert man nämlich Ratten die Nebennieren, dann hört ihr übermässiges Widerstandsvermögen gegenüber Histamin auf (Crivellari 1927, Wyman 1938) und sie gehen im Falle anschliessender Muskelläsion an Verletzungsschock zugrunde (Weiser u. Knott 1939).

Wir führten unsere Versuche auf Grund folgender Annahme durch. Voegtlin u. Dyer gelang es experimentell nicht, bei der Ratte durch mechanische Läsion der Gliedmasse einen traumatischen Schock hervorzurufen. Zufolge der einfachen mechanischen Läsion (Schlagen) geht unzweifelhaft lediglich ein relativ geringer Teil der Zellen zugrunde. Nimmt man an, dass Entwicklung und Schwere des Schocks proportional dem Grade der Zellzerstörung sind, dann ist es denkbar, dass eine Einwirkung, die mit ausgedehntem und hochgradigem Zellzugrundegang verbunden ist, möglicherweise selbst bei der Ratte den Schwellenwert erlangt, der bereits zur Entwicklung von Schock führt. Deshalb wählten wir anstatt Schlagens Gefrieren der Extremitäten in der Annahme, dass letzteres eine erheblich umfangreichere und tiefergreifende Zellzerstörung im Gefolge hat.

VERSUCHE.

Wir führten unsere Versuche an gleichaltrigen weissen männlichen Ratten der gleichen Zucht durch. Wir übergossen den in oberflächlicher Äthernarkose befindlichen Tieren einer Gruppe beide hintere Gliedmassen von der Leistenfurche beginnend mit flüssiger Luft bis sie steinhart gefroren waren. Etwa ½ Stunde nach dieser Prozedur löste sich die Starre der Beine, sie wurden ödematös, die Tiere waren hinfällig, matt, und ein Teil derselben ging binnen 4—8 Stunden nach der Behandlung zugrunde.

Bei der Sektion untersuchten wir vor allem die behandelten Gliedmassen: die Extremität ist in ihrem ganzen Umfange ödematös geschwollen und bläulich-livid verfärbt. Nach Entfernung der Haut

finden sich im subkutanen Bindegewebe, zwischen den Muskeln und in den Muskeln selbst Blutungen, die Muskelfasern sind geschwollen und zerschleisslich. Mikroskopisch (Abb. 1.) ist völliger Zerfall der normalen Zellstruktur feststellbar, was sich in sehr hochgradiger Verminde-

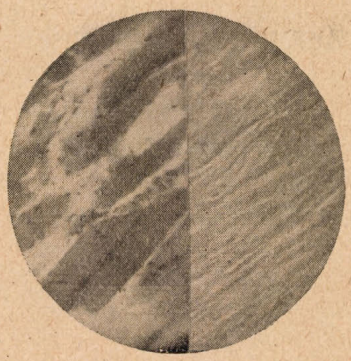

Abb. 1. Mikroskopische Struktur der normalen (links) und der mit flüssiger luft behandelten (rechts) Rattenmuskelegewebe.

rung der Kernzahl und dem Aufhören des normalen Faserverlaufs ausdrückt. Dieser makro- bzw. mikoskopische Befund weist darauf hin, dass in den Weichteilen, vor allem in der Muskulatur zufolge der Be-

TABELLE I.

Eingriff	An-zahl der Tiere	Gewicht in g. der Tiere	Mortalität in den ersten 8 Stdn Anzahl	Mortalität in den ersten 8 Stdn %	Mortalität während 24 Stdn Anzahl	Mortalität während 24 Stdn %
Gefrieren beider hinterer Gliedmassen	19	160.3 ±31.0	8	42.1	9	47.5
Gefrieren nach voraufgehender Abbindung	19	161.9 ±28.7	2	10.5	2	10.5
Abbindung ohne Gefrieren	19	163.1 ±32.6	0	0	1	5.2

handlung mit flüssiger Luft erwartungsgemäss ein hochgradiger und tiefgreifender Gewebszerfall vor sich gegangen ist.

Sodann wollten wir das Ergebnis des Ausschaltens der mit flüssiger Luft behandelten Extremitäten aus dem Kreislauf beobachten. Tre-

ten auch in diesem Falle oben erwähnte schwere Symptome auf, und geht ein grosser Teil der Tiere zugrunde? Dazu banden wir einer anderen Rattengruppe in Äthernarkose beide hintere Gliedmassen in der Inguinalgegend in vollem Umfange ab und übergossen sie sodann mit flüssiger Luft. Auf Tab. I ist ersichtlich, dass Gefrieren nach vorhergehender Unterbindung in einem wesentlich geringeren Prozentsatze Zugrundegehen der behandelten Tiere bewirkte. Wir unterzogen an 19 Ratten die beiden hinteren Gliedmassen der Behandlung mit flüssiger Luft. Binnen 8 der Prozedur folgenden Stunden verendeten 8 Tiere, binnen 24 Stunden aber insgesamt 9 Ratten. Bei ebenfalls 19 Tieren wandten wir die gleiche Behandlung nach Ausschaltung der behandelten Extremitäten aus dem Kreislauf an: binnen der ersten 8 Stunden

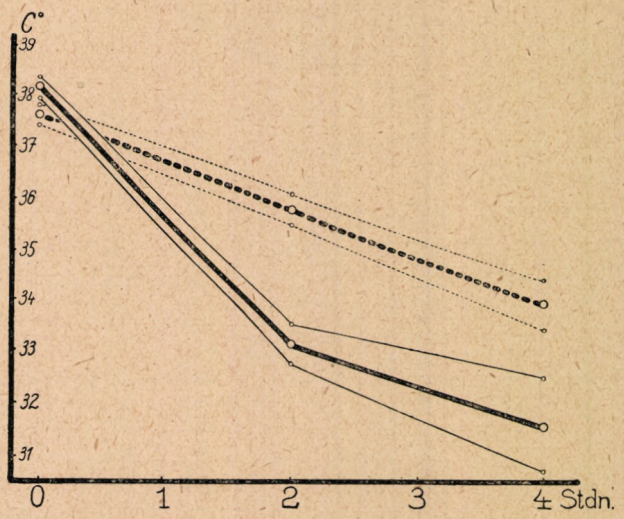

Abb. 2. Rektaltemperatur nach der Behandlung mit flüssiger Luft ohne (ausgezogene Linie) und mit vorangehender Unterbindung der Beine (unterbrochene Linie). Dickere Linien: Mittelwert. Dünnere Linien: einmalige durchschnittliche Streuung.

gingen 2 Tiere ein und binnen 24 Stunden ergab sich kein weiterer Sterbefall. Zwecks Entscheidung, ob etwa das Unterbinden selbst eine grössere Schädigung der Ratte verursache, unterbanden wir 19 Tieren beide hintere Gliedmassen; in dieser Gruppe kam nur 1 Sterbefall vor, u. zw. später als 8 Stunden nach der Behandlung.

Dem Gefrieren der Extremitäten folgend sank auch die Körpertemperatur der Tiere. Abb. 2 zeigt die Rektaltemperatur der Tiere mit nur gefrorenen Beinen (ausgezogene Linie) und der mit solchen nach

voraufgehender Unterbindung (unterbrochene Linie) in der 2. und 4. Stunde nach dem Eingriffe. Die dickeren Linien veranschaulichen die Körpertemperaturmittelwerte mehrerer Tiere, wogegen die von jenen nach oben bzw. unten hin gezogenen Linien das um den Mittelwert befindliche einmalige durchschnittliche Fehlergebiet anzeigen. Zufolge Gefrierens der Gliedmassen sank die Rektaltemperatur der Ratten nach 2 Stunden im Mittelwerte um 5°, nach 4 Stunden um 6.5°. Die Rektaltemperatur nach Unterbindung und darauf folgendem Gefrieren dagegen fiel nach 2 Stunden um 1.5°, nach 4 Stunden um 3,6°. Aus der Abbildung geht zweierlei deutlich hervor: 1. Dass die Behandlung der Extremitä-

TABELLE II.

Raumverhältnis der geformten Elemente und des Plasmas bei Ratten mit gefrorenen Gliedmassen ohne und mit voraufgehender Abbindung 4 Stdn. nach dem Eingriffe. (Hämatokritwert).

Gefrieren ohne Abbindung		Gefrieren mit voraufgehender Abbindung	
Anfang	Nach 4 Stdn.	Anfang	Nach 4 Stdn.
35	32	30	25
36	46	31	30
36	35	30	31
36	40	30	29
35	46	28	24
31	40	28	26
37	47	27	27
35	42	33	35
36	42	35	26
		31	35
		34	28
Mittel: 34.9	41.1	Mittel: 30.6	28.7
	sD = + 3.6		sD = — 1.4

ten mit flüssiger Luft eine sehr hochgradige Körpertemperatursenkung zur Folge hat, 2. dass die Temperaturabnahme durch Unterbindung der gefrorenen Gliedmasse sich erheblich vermindern lässt.

Bei den auf beiderlei Weise behandelten Rattengruppen untersuchten wir im Blute auch das Verhältnis der geformten Elemente und des Plasmas (Tab. II). Wir entnahmen Blut aus den Schwänzen von 9 Ratten und bestimmten im Hämatokrit das Volumenverhältnis der geformten Elemente und des Plasmas. Der Hämatokritwert ergab sich im Mittelwerte der 9 Versuche mit 34,9. Sodann liessen wir die hinteren Gliedmassen der Tiere durch flüssige Luft gefrieren und führten 4

Stunden nach dem Gefrieren eine neue Hämatokritwertbestimmung durch. Dieser wurde in diesem Falle grösser, er betrug 41,1 im Mittelwerte. Signifikante Differenz der beiden Werte = + 3,6. Auf die gleiche Weise verfuhren wir mit 11 Ratten, deren Gliedmassen vor dem Gefrieren unterbunden wurden. Bei diesen Tieren war das Volumenverhältnis der geformten Elemente zum Plasma zu Versuchsbeginn 30.6 im Mittelwert; 4 Stunden nach dem Eingriffe war es nicht grösser: im Mittelwert 28.7 und nahm — wenn auch nicht in signifikanter Weise (s. D. = — 1, 4) — etwas ab. Zufolge Gefrierens der Gliedmassen war also das Blut der Ratten eingedickt, demgegenüber trat bei Ausschalten der gefrorenen Extremität aus dem Kreislauf keine Eindickung, vielmehr eher geringfügige Verdünnung des Blutes ein.

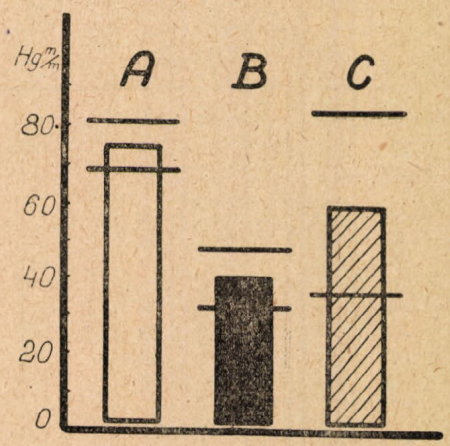

Abb. 3. Blutdruck von normalen (Säule A) und mit flüssiger Luft behandelten Ratten ohne (Säule B) und mit vorangehender Unterbindung (Säule C) der Beine. Die Säulen bedeuten die Mittelwerte, die Linien die einmalige mittlere Streuung.

Wir bemühten uns die zufolge Gefrierens entstehende Blutdruckänderung wie folgt festzustellen. Wir eröffneten in oberflächlicher Ätharnarkose die Bauchhöhle der Ratten, stachen eine Pravaznadel in die Bauchaorta ein, verbanden die Nadel unter Einschaltung einer physiologischen Na-Citratlösung mit einem Hg-Manometer und lasen den Blutdruck unmittelbar ab.

Auf diese Weise bestimmten wir den Blutdruck von 3 Rattengruppen. In Abb. 3 bedeutet Säule A den Blutdruck von 10 unbehandelten Tieren. Dieser betrug im Mittelwerte 75 Hgmm mit ± 5,9 Hgmm Durchschnittsstreuung. Säule B (schwarz) stellt den Blutdruck von 9 Ratten mit gefrorenen Gliedmassen dar. Er betrug im Mittelwert 40 Hgmm mit

7.9 Hgmm Durchschnittsstreuung. Säule C (schraffiert) zeigt den Mittelwert von 11 Tieren mit 60 Hgmm Blutdruck und 24 Hgmm durchschnittlicher Streuung. Wir unterbanden beide hintere Extremitäten dieser in Gruppe C gehörenden Ratten und liessen die Gliedmassen danach gefrieren. Wir berechneten die sign. Differenz zwischen den 3 Mittelwerten. Sie ist zwischen den Blutdrucken der unbehandelten Gruppe und der mit gefrorenen Extremitäten unzweifelhaft, nämlich 9.4, dadegen ist sie zwischen den Blutdrucken von Gruppe A und C, bzw. B und C nicht sicher, sondern lediglich wahrscheinlich, nämlich 2.2 bzw. 2.9.

BESPRECHUNG DER VERSUCHSERGEBNISSE.

Auf Behandlung mit flüssiger Luft hin treten bei weissen Ratten verschiedene krankhafte Veränderungen auf. Diese sind in ihrer Gesamtheit fast identisch mit dem Krankheitsbilde, das den Zustand des traumatischen Schocks charakterisiert.

Der Verletzungsschock weist viererlei Symptome auf, die stets auffindbar und leicht nachweisbar sind (*McNee, Sladden u. McCartney* 1919):

1. Eindickung des Blutes,
2. Blutdrucksenkung,
3. Abnahme der Körpertemperatur,
4. Häufiger tödlicher Ausgang.

In unseren Versuchen beobachteten wir bei Ratten auf deren Behandung mit flüssiger Luft hin die Entwicklung aller 4 Symptome. Die Eindickung des Blutes wurde von der Steigerung des Hämatokritwertes erwiesen. Bei Versuchsbeginn zeigte das Rattenblut 34, 9% geformte Elemente im Mittelwert, wogegen dieser 4 Stunden nach Gefrierbehandlung der Gliedmassen auf 41, 1% anstieg. Die Blutdrucksenkung mit flüssiger Luft behandelter Tiere ist ausgeprägt. Der bei der normalen Rattengruppe mit 75 Hgmm gemessene Blutdruck ging 4 Stunden nach Gefrierbehandlung dieser anderen Gruppe auf 40 Hgmm nach unten, mit einer sign. Differenz von 9, 4 gegenüber dem Normalen. Auch die Rektaltemperatur der Ratten erfuhr eine hochgradige Senkung auf Behandlung der Extramitäten mit flüssiger Luft hin. Auf Grund des unzweifelhalten Vorhandenseins aller 3 ersten obigen Symptome ist also feststellbar, dass zufolge Gefrierbehandlung der Gliedmassen mit flüssiger Luft bei der weissen Ratte ein traumatischer Schock sich entwickelt. Unzweifelhaft ist auch die grosse Sterblichkeit der Tiere eine Folge des Schocks.

Eines der Ergebnisse unserer Versuche ist demnach, dass es uns im Gegensatze zu den bisherigen vergeblichen Versuchen gelang, bei weissen Ratten in jeden Zweifel ausschliessender Weise einen experimentellen Verletzungsschock hervorzubringen. Scheinbar ist dieses Ergebnis Folge der eine grössere Zellzerstörung verursachenden Einwirkung. Bekanntlich vermag sich beim Hunde durch starke und auf grosser Fläche angewandter Kälteeinwirkung ein Schock zu entwickeln (Harkins 1934). Die nähere Ursache liegt sowohl nach den Untersuchungen am Hunde wie in unseren Versuchen in dem von Kälte bewirkten hochgradigen Zellzerfall. Auf Abb. 1 ist klar ersichtlich, dass die Muskulatur zufolge Gefrierbehandlung mit flüssiger Luft weitgehend zugrunde gegangen ist. Intakte Kerne sind kaum zu sehen, und auch der Faserverlauf ist regelwidrig, es befinden sich in ihm viel Kontinuitätsdefekte.

Da die weisse Ratte Schock gegenüber weniger empfindlich ist, so löst der Zerfall relativ weniger Zellen keinen traumatischen Schock aus; aus diesem Grunde ist einfache mechanische Läsion, Schlagen der Gliedmassen ergebnislos. Demgegenüber sind mit ausgedehnter Zerstörung vieler Zellen einhergehende Einwirkungen ausreichend, um auch bei der Ratte einen Verletzungsschock zu bewerkstelligen. Danach scheint sich unsere in der Einleitung ausgesprochene theoretische Überlegung als richtig erwiesen zu haben, und tatsächlich ist der Schock umso schwerer je grösser der Zellzerfall ist.

Der Entstehungsmechanismus des traumatischen Schocks ist nicht geklärt. In der Hauptsache stehen zweierlei Ansichten einander gegenüber. Die eine ist die Annahme von Bayliss u. Cannon (1919), dass der Schock sich auf humoralem Wege entwickle. Nach ihnen gelangt aus den zerfallenden Geweben irgendein toxischer Stoff in den Organismus, der die Permeabilität der Gefässe steigernd, zu Bluteindickung, Blutdrucksenkung und Körpertemperaturabnahme führt.

Demgegenüber steht die andere Auffassung, die in erster Linie von O'Shougnessy u. Slome (1935, 1938) vertreten wird. Nach dieser ist die Schockentwicklung ein reflektorischer Vorgang: zufolge maximalen Erregungszustandes der sensiblen Endapparate kämen die Schocksymptome auf reflektorischem Wege zustande.

Neuerdings fanden Hámori, Karády u. Rusznyák (1942) beim Hunde und der Katze, dass der primäre Schock ein nervöser sei, wogegen der sekundäre Schock auf humoralem Wege zustande komme.

Unsere Versuche scheinen mehr die humorale Auffassung zu unterstützen. Unterbanden wir die Tierextremitäten und brachten sie nachher

durch flüssige Luft zum Gefrieren, dann waren die Schocksymptome erheblich geringeren Ausmasses; einzelne, z. B. die Bluteindickung unterblieben sogar völlig, und ein wesentlich kleinerer Anteil der Tiere ging zugrunde. Demnach schützte Unterbindung der lädierten Gliedmasse das Tier vor der Entwicklung des Schocks. In diesem Falle war die gefrorene Extremität vom Kreislaufe völlig ausgeschaltet, wogegen ihre nervöse Verbindung bestehen blieb.

Die Entstehung ausschliesslich nervösen Ursprungs wird noch mehr verunwahrscheinlicht von dem Umstande, dass die Tiere, wenn man ihre Gliedmassen bloss unterbindet und mit flüssiger Luft nicht zum Gefrieren bringt, die Einwirkung überleben, keines von ihnen binnen 8 Stunden zugrunde geht (Tab. I). In solchem Falle ist nicht mit Gewebszerfall zu rechnen, denn es geschah keine Läsion, dagegen besteht zufolge Anoxämie hochgradige Erregung der sensiblen Endapparate. Diese Tiere äussern während 1—2 Stunden sehr grosse Schmerzen, sie sind sehr unruhig, doch entwickelt sich bei ihnen trotz der maximalen Nervenerregung kein Schock.

Unsere vorliegenden Versuche erteilen vorerst keinen Aufschluss darüber, ob es sich, wenn schon der traumatische Schock auf humoralem Wege zustande kommt, um das Hineingeraten irgendeines toxischen Stoffes in den Kreislauf handelt, oder darum, dass die Gefässe im lädierten Gebiete lokal permeabler wurden, ein hochgradiges Ödem auftritt, das Blutverdickung, Blutdrucksenkung zur Folge hat, an der das Tier zugrunde geht (Smith 1928, Blalock 1930). Die gefrorenen Extremitäten unserer Ratten wurden nach dem Auftauen tatsächlich ödematös, doch beschäftigten wir uns nicht eingehender mit diesem Umstande bei vorliegenden Versuchen und bestimmten nicht das Ausmass des Wasserverlustes in die Extremität vom Gesichtspunkte des Wasserhaushaltes des Organismus aus. Auch dies ist eine der Fragen auf dem Gebiete des traumatischen Schocks, die wir, nunmehr im Besitze der besprochenen Methode, an grösserem Tiermaterial statistisch zu beantworten versuchen werden.

ZUSAMMENFASSUNG.

1. Es gelang bei weissen Ratten einen experimentellen Verletzungsschock durch Gefrieren der Gliedmassen mittels flüssiger Luft auszulösen.

2. Dabei waren 3 typischste Symptome des traumatischen Schocks:

Bluteindickung, Blutdrucksenkung und Körpertemperaturabnahme nachweisbar.

3. Zufolge des Schocks verendete ein grosser Teil der Ratten.

4. Unterbindung der gefrorenen Extremitäten geht mit erheblicher Milderung der Symptome und wesentlicher Verminderung der Sterblichkeit einher. Dies dient als Hinweis dafür, dass beim Entstehen des traumatischen Schocks ein humoraler Faktor die Hauptrolle spielt.

5. Die besprochene Methode ist für das statistische Studium der mit Schock zusammenhängenden Fragen an einer grossen Tierzahl geeignet.

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SCHRIFTTUM.

BLALOCK, A. (1930). *Arch. Surg.* **20**, 259.
CANNON, W. B. W. M. BAYLISS. (1919). *Rep. of the Spec. Invest. Comm. on Surgical Shock. Spec. Rep. of med. Res. Comm.* 26.
CRIVELLARI, C. A. (1927). *Amer. J. Physiol.* 81, 414.
HÁMORI A., KARÁDY I. u. RUSZNYÁK I. (1942). *Orvostud. Közl.* **22.**
HARKINS, H. N. (1934). *Proc. Soc. exp. Biol. a Med.* 32, 432.
MCNEE, J. W., A. F. SLADDEN aa J. E. MCCARTNEY. (1919). *Rep. of the Invest. Comm. on Surgical Shock.-Spec. Rep. of med. Res. Comm.* 26, 33.
O'SHAUGNESSY, L. a. D. SLOME. (1935). *Brit. J. Surg.* 22, 589.
„ „ (1938). „ 25, 900.
SMITH, M. J. (1928). *J. of. Pharmacol.* 32, 465.
VOEGTLIN, C. a. H. A. DYER. (1927). *J. of Pharmacol a. exp. Ther.* 24, 101.
WEISER, R. S. a. H. KNOTT. (1939). *Endocrinology* 25. 379.
WYMAN, L. C. (1928). *Amer. J. of. Physiol.* 81, 414.

ÜBER DIE ROLLE DES HISTAMINS IM ZUSTANDEKOMMEN DES SEKUNDÄREN TRAUMATISCHEN SCHOKCKS WEISSER RATTEN.

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Mit 2 Tabellen im Text.
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In unserer vorgehenden Arbeit machten wir mit der Methode bekannt, die es erlaubt, auch bei Ratten einen traumatischen Schock hervorzubringen. Dies geschieht durch Behandlung der hinteren Gliedmasse des Tieres mit flüssiger Luft. Ein Teil der Tiere geht dabei zufolge des Schocks zugrunde. Schalteten wir die gefrorene Extremität durch Unterbindung aus dem Kreislauf aus, dann waren die Schocksymptome milder, unterblieben eventuell, und die Tiere verendeten nicht. Wir sahen also auch bei der Ratte unter Beweis gestellt, dass der sekundäre traumatische Schock nicht auf dem Wege des Nervensystems, sonder dem des Kreislaufs humoral zustande kommt.

Die Untersucher, die auf Grund ihrer Versuchsergebnisse an anderen Tierarten und am Menschen Anhänger der humoralen Auffassung sind, erklären die Entstehung des sekundären Verletzungsschocks auf zweierlei Art. Quenu (1918), Cannon u. Bayliss (1919) Dale, Laidlow u. Richards (1919) sowie die übrigen Mitglieder der englisch-amerikanischen Schockkommission sind der Ansicht, der sekundäre Schock sei eine traumatische Toxämie. An der Läsionsstelle sammelten sich in den zerstörten Geweben Giftstoffe an, die in den Kreislauf geraten und den ganzen Organismus vergiften. Demgegenüber gelang es Smith (1928) nicht, mittels aus der Extremität schockbehafteter Tiere genommenen Blutes in anderen Tieren einen Schock auszulösen.

Blalock (1930 u. 1939) sowie Feldberg u. Mitarbeiter (1937)

* Die Arbeit wurde im Jahre 1943 fertiggestellt, konnte aber wegen der Kriegsverhältnisse nicht veröffentlich werden.

vertreten die Ansicht, unmittelbare Ursache der Entstehung des sekundären Schocks sei das an der Verletzunsstelle auftretende Ödem. Nach dieser Auffassung werden die Blutgefässe in den lädierten Geweben in hohem Masse durchlässig, wodurch sich das Blut eindickt, das Schlagvolumen geringer wird und demzufolge Kreislaufkollaps zustande kommt.

Natürlich bemühten sich die Anhänger der traumatischen Toxämietheorie um die Aufklärung der chemischen Natur des oder der toxischen Stoffe. Nachdem Dale, Laidlow u. Richards (1919) auf die Ähnlichkeit hinwiesen, die zwischen den Symptomen des traumatischen Schocks und des auf Gefässeinspritzung von Histamin eintretenden Schocks besteht, glaubte Bayliss (1919) Histamin oder eine verwandte Verbindung sei der in Frage kommende toxische Stoff. Diese Auffassung schien auch von den Beobachtungen gestützt zu werden, dass u. a. Äthernarkose die Symptome sowohl des Histamin- wie des traumatischen Schocks schwerer gestaltet. (Dale 1919).

Eines der stärksten Argumente der Histamintheorie war indessen unzweifelhaft die Beobachtung, dass es nicht möglich ist bei gegenüber Histamin ausserordentlich resistenten weissen Ratten einen Schock durch mechanische Läsion der Gliedmassen zu bewirken (Voegtlin u. Dyer 1927). Wird der Ratte die Nebenniere exstirpiert, dann hört ihr Widerstandsvermögen gegenüber Histamin auf, und sie wird zugleich empfänglich für Schock (Crivellari, 1927, Weiser u. Knott 1939).

Bei Ratten, die nach in unserer vorhergehenden Arbeit beschriebenen Gefriermethode behandelt wurden, fanden wir ebenso die Symptome des traumatischen Schocks wie sie bei irgendwelchen anderen histaminempfindlichen Tieren auffindbar sind, dies trotz der grossen Resistenz der Ratte gegenüber Histamin. Dieses Ergebnis eröffnet bezüglich der Natur des Schockstoffes mehrerlei neue, interessante Möglichkeiten. Es ist anzunehmen, dass der toxische Stoff nicht Histamin, sondern ein anderes Gift sei, gegenüber dem die Ratte eine grössere Empfindlichkeit als gegenüber Histamin besitzt. Im Versuche Voegtlin u. Dyers mochte dieser Stoff zufolge einfacher mechanischer Läsion in solch geringer Konzentration sich an der Verletzungsstelle angesammelt haben und in den Kreislauf geraten sein, dass er einen Schock nicht auslöste. Dagegen verursachte unsere stärkere Läsion das Freiwerden so viel solchen Stoffes, dass ein Schock bereits eintreten konnte. Die andere Möglichkeit bestünde darin, dass der Schockstoff auch bei der Ratte Histamin ist. Dann wurde im Versuche Voegtlin u. Dyers

zufolge des mechanischen Traumas wohl eine ausreichende Menge Histamin aus den verletzten Geweben frei, doch fielen die gegenüber Histamin unempfindlichen Tiere nicht in Schock, wogegen in unsren Versuchen zufolge weitgehender Gewebssläsion so viel Histamin auftrat, dass die Ratte getötet wurde.

Zur Bereinigung des Problems boten sich untenstehende Versuche von selbst an. Unsere Fragestellung war: 1. Welche Menge Histamin ist erforderlich, damit es intramuskulär gegeben, gewissermassen in Nachahmung der vom lädierten Gebiet angenommenen Resorption, das Tier töte? 2. Wieviel Histamin ist nachweisbar in den Organen der durch die kleinste letale Histamingabe und traumatischen Schock getöteten Ratten im Vergleich mit normalen Rattenorganen? Antworten auf diese Fragen würden zugleich als Aufklärung dienen, ob traumatischer Schock bei der Ratte als Histaminvergiftung anzusehen sei.

VERSUCHE.

Im ersten Teil unserer Versuche bemühten wir uns die kleinste Histamingabe festzustellen, die intramuskulär gegeben bestimmt 50% der Ratten tötet. Die Titration führten wir an gesunden, 130—200 g schweren weissen männlichen Ratten durch. Das Histamin spritzten wir in der Form seines Dichlorhydrates in 10%iger Lösung in die Muskulatur beider hinteren Gliedmassen ein.

Tab I.

Bestimmung der minimalen letalen Dosis (MLD) von Histamin bei intramuskulärer Injektion in weissen Ratten.

Histaminbase eingespritzt mg/g Körpergewicht.	Zahl der behandelten Tiere	Gestorben
0.8	6	0
1.0	6	0
1.2	6	0
1.5	6	0
1.8	6	1
2.0	6	1
2.5	6	2
3.0	6	4

In Tab. I sind die behandelten Tiere eingetragen, die auf Injektion

der einzelnen Histamingaben hin in den ersten 10 Stunden zugrunde gingen. Wie ersichtlich, verendete von 1500 mg Histaminbase pro Körpergewichtskilogramm keine der behandelten Ratten. Von Gaben von 1500—2500 mg/kg. gingen von 6 behandelten Tieren je 1 bzw. 2 zugrunde, und erst eine Dosis von 3000 mg/kg bewirkte Exitus von mehr als der Hälfte der Ratten. Die geringste tödliche Dosis von Histamin intramuskulär gegeben liegt für weisse Ratten demnach bei 2500—3000 mg/kg.

Im Folgenden bemühten wir uns um den Nachweis von Histamin im Organismus intakter, schockbehafteter und mit Histamin vergifteter Ratten. Den Schock brachten wir nach der in unserer vorgehenden Arbeit veröffentlichten Methode durch Gefrieren beider hinterer Extremitäten zustande. Von Histamin aber spritzten wir in Form seines Dichlorhydrates 3000 mg/kg in die Muskulatur der hinteren Gliedmassen ein. Die Organe der schockbehafteten bzw. histaminvergifteten Ratten arbeiteten wir sofort nach dem Verenden auf. Von schockbehafteten Ratten töteten wir mehrere bereits unmittelbar vor dem Exitus. Die intakten Ratten köpften wir nach Genickschlag und liessen sie verbluten.

1—3 g Organ verrieben wir gründlichst mit Quarzsand und bewahrten sie 7—10 Tage in einer 10%igen Lösung von Trichloressigsäure auf. Der Trichloressigsäureextrakt wurde filtriert und der Organbrei auf dem Filter mit je 5 ccm Trichloressigsäurelösung gewaschen. Auszug und Waschflüssigkeht wurden vereinigt und im weiteren nach Barsoum u. Gaddum (1935) bzw. Code u. McDonald (1937) behandelt.

Die histaminartige Wirkung der Auszüge massen wir am Katzenblutdruck. Obgeich uns bekannt ist, dass sich an einem einzigen biologischen Objekt nicht die Genauigkeit der Bestimmung erzielen lässt wie gleichzeitig an mehreren, begnügten wir uns dennoch lediglich mit dem Katzenblutdruck, denn wir behabsichtigten nicht den absoluten Histamingehalt der Organe zu messen, sondern nur darüber Aufschluss zu erhalten, ob die Histaminwirkung des aus Organen schockbehafteter Tiere gewonnenen Auszuges dem Organextrakte normaler oder histaminvergifteter Ratten näher steht.

Die Katzen schläferten wir mittels Aether-Chloralose ein. Einem Teil derselben gaben wir auch 1 mg/kg Atropin, unterliessen dies jedoch später, als wir fanden, dass die einzelnen Auszüge am gleichen Tiere gleiche Blutrucksenkung vor und nach Atropingabe bewirken. Den Blutdruck massen wir in der Carotis und gaben die Auszüge sowie die His-

taminlösungen bekannter Konzentration in Mengen von 0.2 ccm in die V. femoralis. Bei der Titration verfuhren wir auf die Weise, dass wir zunächst eine den Wirkungen von 0.1, 0.5, 1.0 und 5.0 γ Histamin entsprechende asymptotische Konzentrationswirkungskurve aufnahmen und mit dieser die Blutdrucksenkungswirkung der zu untersuchenden Lösungen verglichen. Zwischen die Einspritzungen wurden Pausen von genau 3 Minuten eingelegt (Burn 1928).

Wir drückten die Ergebnisse in Mengen von Histaminbase aus (Tabelle II). Wiederholt betont sei, dass die Tabellenzahlen nicht absolute Histaminmengen darstellen, sondern lediglich dem Vergleiche dienen. Bezüglich des genaueren Histamingehaltes von Rattenorganen haben Martin u. Valenta (1939) Untersuchungen durchgeführt. Sie bestimmten das Histamin auf Grund zweierlei biologischer Methoden. Unsere in normalen Rattenorganen gefundenen Werte weichen nicht wesentlich von denen dieser Verfasser ab.

TABELLE II.

Histamingehalt der Organe normaler, mit Schock behafteter und histaminvergifteter Ratten. (Werte bei Katzenblutdruck.) γ/g Roh organ gewicht.

	Normal				Schockbehaftet						Histaminvergiftet				
	I.	II.	III.	Mv.	I.	II.	III.	IV.	V.	Mv.	I.	II.	III.	IV.	Mv.
Blut	1.1	2.6	3.8	2.2	0.5	1.9	3.1	0.5	—	1.5	6	25	45	180	120
Hirn	15.0	4.0	2.0	7.0	1.0	2.0	1.8	0.5	0.5	1.2	22	8	41	63	33
Lunge	12.0	8.0	8.2	9.4	12.0	8.1	7.2	3.0	1.4	6.3	70	280	80	370	200
Leber	16.0	3.5	1.5	7.0	0.5	2.0	1.2	0.9	0.2	1.0	125	140	160	678	278
Niere	3.2	2.2	0.5	2.0	0.5	1.8	—	6.0	1.2	2.4	430	110	100	375	254
Darm	27.0	16.5	24.5	22 7	14.0	13.5	5.8	2.2	1.6	7.4	200	160	120	315	200
Haut	13.5	26.0	25.0	21.5	4.0	—	19.0	3.0	5.0	7.8	500	250	50	1160	490
Muskel	4.9	1.8	1.8	2.8	0.8	—	7.0	2.8	3.0	3.4	4500	2100	2500	1100	2550

Beim Vergleich des Histamingehalts der Organe normaler, schockbehafteter und histaminvergifteter Tiere fällt zunächst der hohe Histamingehalt der vergifteten Ratten auf. In den Organen dieser Tiere fanden wir etwa das 100fache des normalen Histamingehalts. Im Muskel der vergifteten Tiere stellten wir 1000faches Histamin im Vergleich mit dem Normalen fest. Dies bedeutet offenbar, dass die Base aus der Muskulatur nur teilweise resorbiert wurde, weil wir die Histaminsalzlösung in jedem Falle in die Muskulatur der hinteren Gleidmasse einspritzten, dagegen das Untersuchungsmaterial dem gleichen Muskel entnahmen.

Zwischen den Histamingehalten der Organe normaler bzw. schockbehafteter Tiere fanden wir keine wesentliche Abweichung. Keines-

falls nahm der Histamingehalt der Organe der schockbehafteten Ratten dermassen zu, dass im Falle von Schock Histaminvergiftung angenommen werden könnte.

Besonders interessant ist, wie sehr die Histamingehalte der Muskeln normaler bzw. schockbehafteter Tiere übereinstimmen. Das Untersuchungsmaterial entnahmen wir auch hierbei der hinteren Gliedmasse, die wir im Falle schockbehafteter Ratten mittels flüssiger Luft lädierten, und wo nach Auffassung der Anhäger der Histamintheorie Histamin in erster Linie frei werden solle.

BESPRECHUNG DER VERSUCHSERGEBNISSE.

Es gelang uns zu beweisen, dass traumatischer Schock auch bei der Ratte möglich ist, und dass dieser auf humoralem Wege zustande kommt. Abbindung der Gliedmassen verhinderte nämlich in hohem Masse die Entwicklung schwerer Symptome. Der Umstand, dass es möglich ist, auch bei der gegenüber traumatischen Schock so sehr resistenten Ratte solchen durch Gewebsläsion auf die gleiche Weise auszulösen wie bei sehr schockempfindlichen Tierarten sowie, dass die Schocksymptome von der Verbreitung des Schockgiftes durch den Blutkreislauf im Körper verursacht werden, ist ein neuerer stichhaltiger Beweis für den humoralen Mechanismus der Schockentstehung. Es ist dies gerade jetzt, wo der Meinigungsaustausch über dieser Frage lebhafter geworden ist, von Bedeutung.

Während unsere bisherigen Versuche ausschliessen, dass der Schock auf reflektorischem Wege entstehe wie dies Brown-Sequard meinte, und was durch neuere Beobachtungen wieder einen Anhaltspunkt gewann (O'Shaugnessy u. Slome 1935 u. 1938), schliessen sie aber nicht die Möglichtkeit aus, dass beim humoralen Entstehen Toxämie oder an der Verletzungsstelle zustande kommender Flüssigkeitsverlust wirksam seien (Blalock, Feldberg, Krull u. Schilf l. c.). Wie erwähnt, schwollen die Gliedmassen unserer Ratten nach Gefrieren ödematös an. Allein es ist uns nicht bekannt, ob diese Schwellung dazu ausreicht, dass aus der Gefässbahn so viel Flüssigkeit in die Gewebe ströme um die Eindickung des Blutes, die Kreislaufhinfälligkeit und das Verenden zu erklären. Betont sei, dass der normale Organismus einen vom Blute herrührenden erheblich grösseren Flüssigkeitsverlust, als er in einer solchen ödematösen Extremität erfolgt, unzweifelhalf auszugleichen vermag. Überdies ist unbestreitbar, dass die Stoffe, die in der lädierten Gliedmasse selbst ein Ödem verursachen, die gleiche Wirkung auch anderswo im Körper ausüben werden.

An Hand unserer besprochenen Versuche untersuchten wir eigentlich die Frage, ob beim durch Schock erfolgenden Zugrundegehen von Ratten eine histaminartige Vergiftung wirksam sein könne. Deshalb bestimmten wir den Histamingehalt der Organe von Ratten, die einerseits zufolge Schocks, anderseits an Vergiftung nach Histamineinspritzung verendeten. Die Ergebnisse erwiesen in jeden Zweifel ausschliessender Weise, dass in den Organen der Ratte sich sehr viel Histamin ansammeln muss, damit sie durch solches getötet werde. In den Organen von an Schock zugrunde gegangenen Tieren ist eine solche Histaminanhäufung überhaupt nicht vorhanden. Zwischen den Histamingehalten der Organe normaler bzw. schockbehafteter Ratten besteht kein Unterschied; in beiden Gruppen ist dagegen im Vergleich mit den durch Histamin getöteten Tieren nur der hundertste Teil desselben enthalten. Schock tötet also die weisse Ratte durch ein aus dem lädierten Gewebe frei werdendes und in das Blut geratendes Gift, ohne dass der Histamingehalt in den Rattenorganen sich veränderte. Der bei der Ratte nach Gefrieren sich einstellende traumatische Schock ist demnach keine von aus lädiertem Gebiete stammendem Histamin bewirkte Vergiftung. Einer der Beweise der Theorie, dass das Schockgift Histamin sei, war gerade der Umstand, dass die Ratte gegenüber Histamineinspritzung und traumatischem Schock gleicherweise resistent ist, wogegen andere Tiere gegen beide gleicherweise empfindlich sind, weiterhin, dass Epinephrektomie sowohl bei Ratten wie anderen Tierarten die Resistenz gegenüber Schock bzw. Histamin in gleicher Weise steigert. Bei Berücksichtigung auch unserer Versuchsergebnisse lässt sich aus obigen Tatsachen bereits nicht mehr schliessen, dass das Schockgift Histamin sei. Nach unseren Versuchen nämlich wurde die Ratte traumatischem Schock gegenüber nicht deshalb für resistenter befunden, weil hierbei — obgleich auch in diesem Zustande Histamin bei der Läsion frei wurde, und die Ratte gegenüber Histamin widerstandfähiger ist — sich kein Schock entwickelte, sondern weil die Gewebe nicht genügend lädiert waren, damit das zur Entstehung des Schocks erforderliche Schockgift in ausreichender Menge produziert werde. Ist die Läsion — wie in unseren Versuchen — ziemlich gründlich, dann wird auch bei der Ratte eine entsprechende Menge Schockgift frei, und gelangt dieses in erforderlichem Quantum ins Blut, dann geht auch die Ratte an Schock zugrunde. Andererseits beweisen unsere Versuchsergebnisse auch, dass dieser Schokstoff bei der Ratte nicht das aus dem lädierten Gewebe stammende Histamin sein kann. Einer der Gründe der grösseren Schock-

resistenz bei der Ratte ist also der bessere Widerstand der Gewebe gegen Läsion, mit der zufälligerweise zugleich grössere Resistenz gegenüber Histamin vorkommt. Auf Epinephrektomie hin nimmt demnach die Schockempfindlichkeit der Ratte nicht aus dem Grunde zu, weil ihr Widerstand gegenüber Histamin geringer wird, sondern weil Epinephrektomie auch die Resistenz gegenüber dem Schockstoff, der nicht Histamin ist, herabmindert.

Im übrigen spricht ausser unseren unmittelbar beweisenden Versuchsergebnissen auch folgende theoretische Überlegung dagegen, dass das Schockgift der Ratte Histamin wäre. Nimmt man an, dass 20% der Rattenmuskulatur Eiweiss sind, wogegen etwa 3% davon auf Histidin entfallen (Beck u. Casper 1928), dann müssten sich, damit zur Tötung einer 200 g schweren Ratte asureichende 500 mg Histamin entstehen, 700 mg Histidin decarboxylieren, was den Abbau von 23 g Eiweiss, also etwa 115 g Rattenmuskel erforderte. So viel beträgt aber nicht einmal die ganze Körpermuskulatur der Ratte.

Es sei darauf aufmerksam gemacht, dass die Hypothese, nach der das Schockgift Histamin sei, nicht allein für die gegenüber Histamin resistente Ratte an Beweismitteln auf Grund unseres Versuches verloren hat, sondern auch zufolge der Versuchsergebnisse anderer Verfasser auch bezüglich histaminempfindlicher Tiere. Wohl fand Minard (1937) im aus den traumatisierten Gliedmassen von Hunden abgehenden Blute um 80% mehr Histamin als im venösen Blute der intakten Extremität, demgegenüber führten aber McDonald u. Woolfe (1938) Histaminbestimmungen im abfliessenden Blute der traumatisierten bzw. intakten Gliedmassen von 14 Katzen durch und fanden dabei solch geringe Unterschiede, die auf keinen Fall Urheber der schweren Schocksymptome sein können. Also auch bei histaminempfindlichen Tieren ist es zweifelhaft, ob die Symptome des sekundären traumatischen Schocks zu Lasten des Histamins zu buchen seien; bei weissen Ratten aber ist die Histaminvergiftung bestimmt auszuschliessen.

Ein weiteres überraschendes Ergebnis unserer Versuche ist, wie gross sich bei intramuskulärer Dosierung die tödliche Histamingabe der weissen Ratte stellt. Die geringste tödliche Gabe pro Körpergwichtskilogramm wird angegeben von Voegtlin u. Dyer (1927) mit i. v. 900 mg Histaminphosphat, von Crivellari (1927) mit 500—700 desgleichen, von Marmorston-Gottesmann u. Gottesmann (1928) i. v. und i. p. mit 1600 mg. desgleichen, von Schmidt u. Stähelin (1929) mit i. v. 230—270 mg. Histaminchlorhydrat, wogegen nach Wyman (1928) das Tier i. p. 1000 mg/kg. Histaminphos-

phat verträgt. Wir haben dies in unseren Versuchen mit 2500—3000 mg/kg Histaminbase festgestellt, was 6000—8300 mg Diphosphat entspricht. Dies bedeutet, dass die geringste tödliche Dosis intramuskulär gegebenen Histamins etwa 4—5-mal so gross ist wie die mitgeteilten grössten i. p. und i. v. Dosen.

Bevor jedoch daraus weitergehendere Schlussfolgerungen abgeleitet werden, muss man berücksichtigen, dass wir in der Gliedmassenmuskulatur der histaminvergifteten Tiere 1.1—4.5 mg/g Histamin fanden, was ein Hinweis auf die schlechte Resorption des Histaminsalzes aus der Muskulatur ist. Berücksichtigt man den raschen Abbau des Histamins im Organismus, so wird es auf Grund des obigen verständlich, dass die Ratte intramuskulär tatsächlich eine sehr grosse Histamingabe zu ertragen vermag.

ZUSAMMENFASSUNG.

1. Wir spritzten in den hinteren Gliedmassenmuskel 130—200 g. schwerer männlicher weisser Ratten Histamin ein. Es verendeten mehr als 50% der Tiere, wenn wir 2500—3000 mg pro Körpergewichtskilogramm verabreichten.

2. Wir bereiteten Auszüge aus den Organen normaler, bzw. durch Gefrieren schockbehafteter bzw. mit Histamin vergifteter Ratten und untersuchten die Wirkung der Extrakte auf den Katzenblutdruck.

3. Der Histamingehalt der Organe der histaminvergifteten Tiere ist etwa 100mal grösser als der normaler Ratten. Zwischen den Histamingehalten der Organe normaler bzw. gefrierschockbehafteter Tiere besteht kein mittels der angewandten Methode nachweisbarer Unterschied.

4. Auf Grund der Ergebnisse müssen wir die Auffassung ablehnen, nach der bei weissen Ratten die Ursache des sekundären Schocks Histaminvergiftung sei.

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SCHRIFTTUM.

Barsoum, G. S. a. J. H. Gaddum. *J. of. Physiol.* 85, 1.
Bayliss, W. M. (1919). *Spec. Rep. of med. Res. Com.* 26, 3.
Beck, K. u. E. Casper (1928). *Z. untersuch. Lebensmitt.* 56, 437 (Zitiert aus Abderhalden: Bioch. Handlexicon Bd. 14, 210.

der weissen Ratte stellt. Die gerinste tödliche Gabe pro Körpergwichts-

BLALOCK, A. (1930). *Arch. Surg.* 20, 259.
„ (1939). *Surg. Gynecol a. Obstetr.* 68, 278.
BURN, J. H. (1928). *Methods of Biol. Assay*, Edinburgh.
CANNON, W. B. a. W. M. BAYLISS (1919). *Rep. of the Spec. Invest. Comm. on Surgical Shock. Spec. Rep. of med. Res. Comm.* 26.
CODE, O. F. a A. D. MCDONALD. (1937). *Lancet* 2, 730.
CRIVELLARI, C. A. (1927). *Amer. J. Physiol.* 81, 414.
DALE, H. H (1919). *Spec. Rep. of med. Res. Comm.* 26, 15.
„ P. P. Laidlow a. A. N. Richards (1919). *Spec. Rep. of med. Res. Comm.* 26, 8.
FELDBERG, W. u. E. SCHILF. (1937). *Histamin*, Berlin.
MARMORSTON-GOTTESMANN a. J. GOTTESMANN. (1928). *J. exper. Med.* 97, 503.
MARTIN, J. u. A. VALENTA. (1939). *Arch. Exp. Pathol. u. Pharmacol.* 193, 303.
MCDONALD, A. D. a. G. WOOLFE. (1938). *J. Physiol.* 93, 58.
MINARD, D (1937). *Amer. J. Physiol.* 119, 375.
QUENU. (1918). *Rev. de Chir.* 56, 204.
O'SHAUGNESSY, L. a. M. SLOME. (1935). *Brit J. Surg.* 22, 589.
„ „ (1938). „ 25, 900.
SCHMIDT, G. W. u. A. STÄHELIN. (1929). *Z. f. Immfrschg.* 60, 222.
SMITH, M. J. (1928). *J. of Pharmacol* 32, 465.
VOEGTLIN, C. a. H. A. DYER. (1927). *J. of Pharmacol.* 24, 101.
WEISER, R. S. a. C. H. KNOTT. (1939). *Endocrinology.* 25, 379.
WYMAN, L. C. (1928). *Amer. J. Physiol.* 87, 29.

BLOOD PRESSURE RESPONSE TO HISTAMINE IN CATS, BEFORE AND AFTER PRE-TREATMENT WITH HISTAMINE.

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Tachyphylaxia (Führer, 1912) and histamine desensibilization (H. D.) (Karády, 1936, Rusznyák, Karády, Szabó 1934, 1935) diminish the observed concentration effect curve of histamine on arterial blood pressure. Between the 2 diminishing effects the essentially significant difference is that in histamine tachyphylaxia constantly increasing doses must be given, in intervals of seconds, to cause a smaller blood pressure diminution; H. D. consists of injecting small preliminary doses of histamine once or more per day, over a period of 8—10 days. Control experiments could not confirm that such pre-treatment brought about a lessening in blood pressure diminution. (Kokas, Sarkady, Went, 1938).

The contradiction probably arises from the fact that the response of blood pressure after the same doses shows not only individual variation but, in the same animal, in the same experiment, gives visibly different results. It would therefore only be possible to resolve the problem if there were large groups both of desensibilized and control animals. As the contradiction exists in both schools of thought, both of which worked with small numbers of animals, of those experiments it can only be said that the H. D. does not cause a greatly significant difference in the blood pressure response following on single doses, beyond a large variation.

It would be easier to decide the question if we compared, not groups of animals, but the reactions of the same animal normally and after H. D. The equality of the response is further raised if in place of the response following single H doses we compare concentration

effect curves in which the doses of histamine given were proportioned to each other as 1:10:100.

In this work, with this procedure, we proved that in cats the H. D. does not influence the arterial blood pressure histamine concentration curve. We did not investigate the tachyphylaxic question in this experiment. FÜHNER, in experiments mentioned, seems to have solved the question.

THE EXPERIMENTS.

For the experiments we used 9 cats, of both sexes, weighing 1.5—2.5 kg. The measuring of the blood pressure took place under ether-chloralose narcosis, in such a way that 0.05 g. chloralose per kg. body weight was injected in the veins, and the superficial narcosis deepened

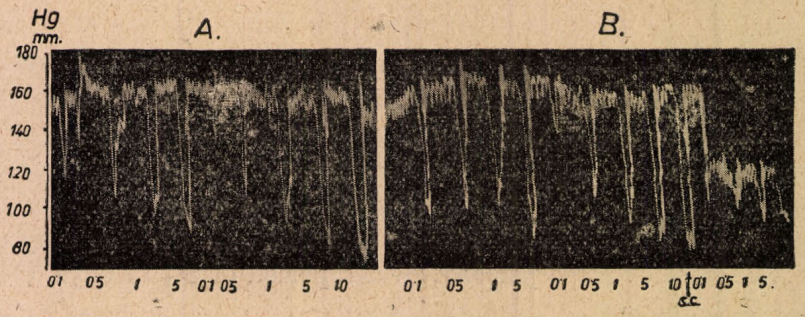

Figure 1. The blood-pressure-diminishing effect of histamine-doses (0.1—10.0 μg) in the same cat before (A) and after (B) 14 days pre-treatment with histamine. At sc. 1 mg histamine was given subcutaneously.

through the constant inhalation of a little ether. In the first experiment, under sterile conditions, we prepared one of the femoral arteries, introduced a canule into it, and registered the blood pressure with the aid of a mercury manometer on a slowly turning kymograph. We injected the histamine in dichlorhydrate form dissolved in a 0.2 ml. physiological salt solution in the femoral vein. We gave the injections at exact 3 minute intervals (BURN 1928). 0.1, 0.5, 1.0, 5.0 and 10 μg. histamine bases were injected one after the other. We got the concentration effect curve through the consequent registration of the blood pressure. After the experiment we removed the canule from the artery, bound up the artery, treated the wound with ultaresptyl-urea powder, and sewed it up.

In the time between the first and second experiments each animal received 3 daily subcutaneous injections of 1—1 mg. histamine of 0.1% solution in its dichlorhydrate form. The effect of the injections on the animals we observed to be as follows: 1—2 minutes after the injection lassitude and weakness, with slavering and in some cases vomiting. These symptoms lasted about 20—30 minutes, after which the cats drank abundantly.

12—19 days after first measuring the blood pressure we again narcotized the animals in the above manner. On this occasion the blood pressure was measured in the carotis and the concentration effect curve taken with histamine injection at 3 minute intervals, exactly as in the first case. Figure 1 shows the graph of some of the concentration effect curves.

In some experiments, after the graphing of the concentration effect curve we injected the animals subcutaneously with 1 mg histamine, upon which the blood pressure dropped and for a long time remained at a low level. If meanwhile we gave extra doses of histamine intravenously we got much less fall in blood pressure (Figure 1, after s. c. sign).

EXPERIMENTAL RESULTS AND DISCUSSION.

Figure 2 sums up the results of the experiment. In the Figure we show the concentration effect curve of each animal before treatment (straight line) and that after long treatment with histamine (broken line). In some animals both before and after treatment we took only one effect curve, in others two. In this case the mean values appear in the Figure.

The first conspicuous thing about the Figure is that in those experiments where we registered 2 effect curves one after the other, the blood pressure response following on the intravenous histamine administration varied in the same animal. The difference could be as much as 20%. On this difference the increase in dose had no effect. Table I. is a comparison of the concentration effect curve of several animals, and shows the individual variations within the species. In respect to our principal question, whether H. D. brought about a change in the blood pressure, it appeared from our experiments that in not a single animal did the blood pressure fall less after the histamine treatment than before. As far as we can speak of a change in the reactions we must say that a slight degree of increase in the fall of

blood pressure took place, principally in the case of the 0.1 μg doses.

To get an appreciable picture of the consequence of the histamine treatment, in spite of the individual and group variations, we made a statistical valuation of the change in blood pressure fall. In Table I. we have assembled the mean blood pressure values of the single histamine doses, before and after treatment. We reckoned the significant difference between the 2 mean values in the usual way. It appears from the Table that the significant difference between the mean blood pressure values and those effected by the 0.5, 1.0, 5.0 and 10 μg.

TABLE I.
Statistical evaluation of the experimental results.

Histamine dose μg.	Fall in blood pressure in Hgmm. Pre-treated	Untreated	Significant difference between the blood pressure fall in untreated and treated animals.
0.1	17.5 ± 8.4	27.5 ± 12.0	+ 2.75
0.5	35.8 ± 9.53	39.5 ± 12.53	+ 0.91
1.0	43.8 ± 10.9	50.0 ± 10.2	+ 1.45
5.0	59.7 ± 11.4	62.8 ± 11.1	+ 0.67
10.0	71.0 ± 11.28	68.6 ± 17.2	— 0.27

histamine is smaller than 2; that is the amount was exactly the same in the treated and untreated animals. In the untreated animals after a 0.1 μg intravenous histamine injection the blood pressure fell by a mean value of 17.5 Hg. mm., after treatment by 27.5 Hg. mm. The 2.75 significant difference between the two values makes improbable the complete equality; that is, a slight degree more sensibility can be remarked after protracted treatment with histamine on the basis of 0.1 μg intravenous injections.

The fall in blood pressure brought about by intravenous histamine injection is in part the result of the fact that the base widens the arterioles and praecapillaries, besides which it changes the irritability of the centres, inasmuch as, through the blood thickening, the quantity of liquid circulating diminishes.

These effects were also brought about if histamine — in larger doses — was injected subcutaneously. This condition we could observe directly (Figure 1, after s. c.) but we could confirm it indirectly as well, in that after the subcutaneous injection the animals were weak and languid, and thirsty due to the thickening of the blood. In our experiments, after making the first histamine concentration effect

curve, we daily gave the animals 1—1 mg. subcutaneous injections for a period of two weeks. In spite of the fact that during the time beween taking the two effect curves the histamine did cause a fall in blood pressure in the cats on 40—50 occasions, we did not find a diminished sensibility to histamine.

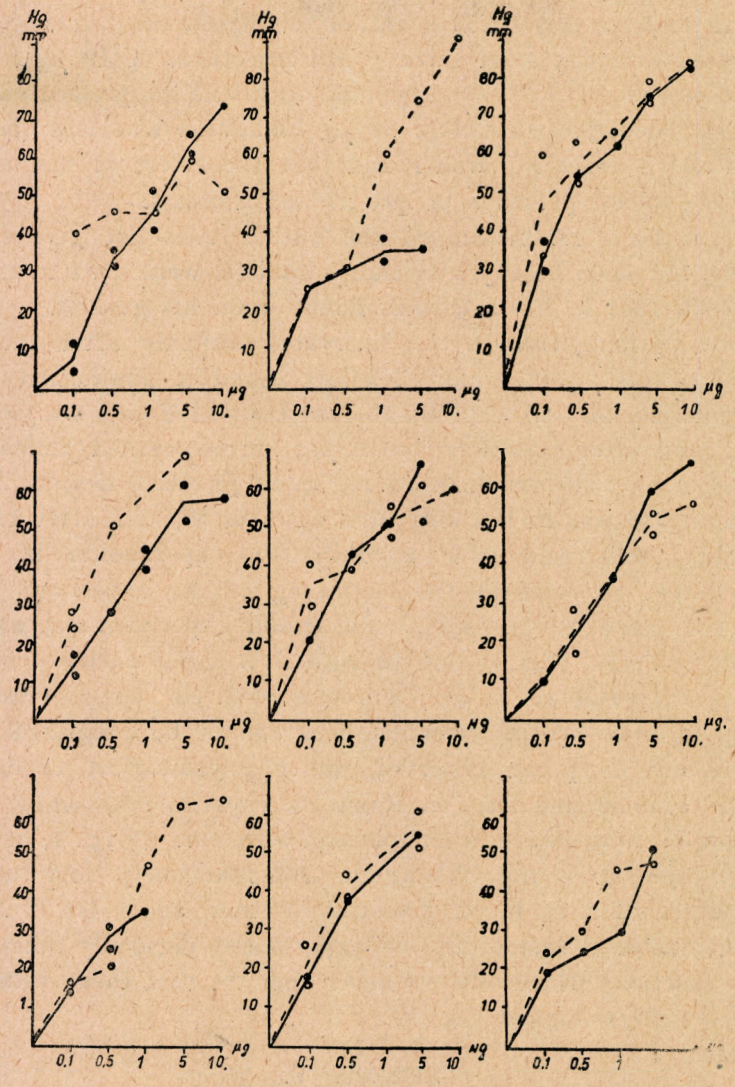

Figure 2. The effect of different histamine-doses in diminishing the blood-pressure, in 9 cats, before (straight line) and after (broken line) pre-treatment. Abscissa: histamine doses given intravenously; ordinata: fall of the blood-pressure in Hgmm. after the histamine doses.

From our experiments we draw the conclusion that cats cannot be desensibilized by lengthy histamine treatments with the effect of reducing blood pressure by histamine administered intravenously. Those mechanisms which cause a fall in blood pressure do not change, even in consequence of long training.

To terminate, we must criticize KARÁDY's experiments (KARÁDY 1936) a little more closely. This author announces the following results: In untreated animals, after intravenous injections of 10 μg histamine, the blood pressure fell 20—40 Hg. mm. In histamine pretreated animals the fall in blood pressure after 10 μg histamine was less (5—10 Hg. mm); in a few cases he even found that the effect of histamine was to raise the blood pressure of pre-treated animals.

But in the work mentioned the author does not give statistical valuations, nor does he say how many animals were used in the experiments. This fact in itself throws doubt upon his results.

It is surprising that in the untreated animals after intravenous injections of 10 μg histamine KARÁDY found a diminution of blood pressure of only 20—40 Hg. mm. In our untreated cats we got a mean 71.0 Hg. mm. after injections of 10 μg, in the treated animals a fall of 68.6 Hg. mm. The smallest of all blood pressure descent following 10 μg histamine was in the untreated animals 58, and, after treatment, 52 Hg. mm. Also remarkable in KARÁDY's experiments is that his animals' blood pressure at the beginning of the experiment was extremely low: about 80 Hg. mm, while in our experiments it was around 120—160 Hg. mm. This is due to KARÁDY's using partly decerebrated animals, partly animals in deep narcosis with cut vagus. Observations made under these special circumstances cannot be compared with the blood pressure response observed under physiological conditions. It should be remembered that in KARÁDY's experiments, while he began with a low blood pressure in comparison with ours (Fig. 1, after s. c.), when we injected 1 mg. histamine subcutaneously and under the lengthy diminution in blood pressure — then about 80 Hg. mm. — again administered histamine, we experienced decidedly smaller blood pressure reactions. Naturally we never thought that these values should be taken as physiological values.

SUMMARY.

We measured the fall in blood pressure of 9 cats in sterile conditions on injecting 0.1, 0.5, 1.0, 5.0 and 10 μg histamine intravenously. Thereafter for 12—19 days we gave the animals 3 daily subcutaneous

injections of 1—1 mg. histamine. After the treatment we again measured the histamine concentration effect curve of the arterial blood pressure.

There is no change in sensibility before and after treatment.

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REFERENCES.

BURN, J. H. (1928). *Methods of Biol. Assay.* Edinburgh.
FÜHNER, H., (1912). *Münch. Med. Wchschr.* 59, 852.
KARÁDY, St. (1936), *Arch. exp. Pathol. u. Pharmakol.* 180, 283.
KOKAS F., SARKADY L., WENT I. (1938). *Magy. Orv. Arch.* 39, 408,
RUSZNYÁK I., KARÁDY L., SZABÓ (1934), *Orv. Hetilap* 14. — *Deutsch. Med. Wchschr* 60, 1670.
„ „ „ (1935). *Deutsch. Med. Wchschr.* 61, 1111.

THE DISTRIBUTION OF LETHAL SENSITIVITY TO HISTAMINE IN NORMAL AND HISTAMINE PRE-TREATED GUINEA PIGS.

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In 1934 Rusznyák, Karády, Szabó, and Karády and Bentsáth in 1935 reported that repeated intravenous histamine (H) injections (HT) act as a preventive in postoperative shock in certain sensitive human patients. To denote this kind of histamine treatment these investigators coined the expression: "histamine desensitization."

From this observation, research branched in two directions. One of them was the critial repetition on human patients of the original clinical observation (Bretschger (1936), Rusznyák, et al., 1936; Milkó, 1936), the other a series of animal experiments in order to furnish more decisive evidence (Karády, 1936, MacKay and Clark, 1938, Karády, 1938.)

The underlying hypothesis of "histamine desensitization" was that repeated injections of small doses of histamine enable the organism, through some unknown mechanism, to withstand larger amounts of this substance supposed to be released according to the Bayliss and Cannon shock-hypothesis. By surgical statistics the question of the development of such a histamine desensitization cannot be decided. Even if it were proven that surgical shock is a H poisoning — which it is not — the differences between surgical shock in man and H poisoning in different animals are so great and so numerous that the two should be subjected to separate investigations. In the case of H desensitization in man against traumatic shock, the question can be decided only with very large human material, because a) in this case the "trauma" cannot be

administered as exactly as the H can and there is therefore no fixed scale to which the frequency of mortality may be referred; b) the individual variations of sensitivity in man are very great.

The question whether pre-treatment with small doses of H increases the resistance against larger doses given at a later occasion can be decided only by comparing the resistance of non-treated and treated animals to H. Such attempts have already been made. Thus HORTON, MACLEAN and CRAIG (1929) found that 50% of the desensitized animals survived a H dose killing 87% of the controls. CLARK and MACKAY (1939) found that the survival time after the injection of 0.5—0.6 mg/100 mg b. w. is not greater in the pre-treated animals than in the controls. FARMER (1939, a and b) finds an increased resistance to histamine after both s. c. and peroral H treatment. BARLOW and HOMBURGER (1941), on the other hand, arrived at negative results, as did KOKAS, SARKADY and WENT (1938). These experiments, however, all fall victim to the same criticism: They cannot be subjected to statistical testing, and without this they do not warrant any conclusion.

The correctness of this attitude is easily proved. SCHENK (1922) gives 350 μg/100 g. b. w. as the MLD for s. c. injections in the guinea pig. SCHMIDT and STÄHLIN (1929) report that some guinea pigs succumb only to 1000 μg/100 g b. w. When lethal sensitivities differing as widely as 1 to 3 occur in a population, as in this case, differences of 50—100% in the mortalities, and still less in survival time, found on comparing two groups, generally of small number, are meaningless. So long as the comparison of the HT and control animals is not made on an indefinitely large population and with the knowledge of the standard deviation of the two sets of results, the question whether the occurrence of some apparently more resistant animals in the HT group was not merely a chance effect, cannot be decided. TREVAN (1927) developed a method of comparative measurement of the sensitivity of two groups of animals to the same drug. His method fulfills the requirements of variation statistics. A similar, improved, method was elaborated by GADDUM (1933). The statistical analysis in our work consists in the comparison of the entire "characteristic curves" of the two populations, of normal and histamine pre-treated (HT) animals. The characteristic curve is a frequency curve in which corresponding percentages of mortalities are plotted against several doses, causing mortalities between 0 and 100% (TREVAN l. c.). The shape and slope of such curves are characteristic of the species and the drug applied. Whether the differences in the scale of the doses between two characteristic curves is

merely due to the variation of sampling or is outside the limits of same, can be determined — in the case of normal distribution — by the calculation of the significance of difference between the average lethal doses (ALD) of the two groups. The ALD is that amount of drug which kills 50% of an indefinitely large number of animals. It is usually expressed in mg/kg.

The standard deviation must be kept as low as possible. Variations of the lethal sensitivity are often due to variations in the experimental circumstances, such as: the sex, weight, age of the animal, the site of the injection, the amount, the pH, the temperature of the solvent, the rate of the injection, etc., and not to a variation of the sensitivity. Proper precautions must therefore be taken to standardize in the two groups compared all experimental circumstance of which an influence is known.

Since the earlier experiments quoted were made without due regard for these aspects, neither the affirmative nor the negative results are satisfactory. SMITH-KARÁDY (1940) made an attempt to produce more evidence. Of her results, as will be shown below, only the intra-cardiac (i. c.) injection experiments are statistically conclusive.

In order to decide the alleged question of an H desensitization against H, we therefore determined the "characteristic curve" for H in normal and H pre-treated guinea pigs of both sexes and compared them statistically.

METHODS.

For the comparison of the death-rate in normal and HT animals, young guinea pigs 312 ± 68 g of both sexes, divided into groups according to sex, were used. All the animals lived in the same climatically conditioned room (25° C) and received the same diet for at least 1 month before the experiment. This consisted of oats, carrots (yellow and white), alfalfa, and fresh green stuff, but no milk or water.

Table I shows the groups formed of the animals. As is seen in this Table altogether 321 male and 80 female animals were used. The experimental period extended, with long interruptions, from October 1941 to November 1946.

The HT was carried out on the 5 Groups VI, VII, VIII, IX and X, as shown in Figure I. The HT of all the groups began with a dose of 5, 10, or 40 μg/100 g. b. w. H (crystalline dichlorhydrate dis-

TABLE I.

The groups of guinea-pigs in the death-rate comparison experiments.

Nr.	HT Treatment	Site of injection	Site of testing injection	Total Nr of animals.	Sex	BW g.	Time of experiment
I.	none	—	s. c.	83	male	287	1. VI. 43—28. VIIII. 43.
II.	none	—	s. c.	34	male	225	XII. 1941.
III.	none	—	i. p.	95	male	235	25. III. 43—29. IV. 43.
IV.	none	—	i. p.	12	male	385	XII. 1941.
V.	none	—	i. p.	40	female	302	XI. 1943.
VI.	short	s. c.	s. c.	40	male	251	1. VI. 43—28. VII. 43.
VII.	short	s. c.	s. c.	24	male	385	6. III. 1942.
VIII.	short	s. c.	i. p.	13	male	385	6. III. 1942.
IX.	long	s. c.	s. c.	40	female	302	XI. XII. 1943.
X.	short	s. c.	s. c.	20	male	369	13. X. 46—7. XI. 46.
				401		312.1 ± 68.4	

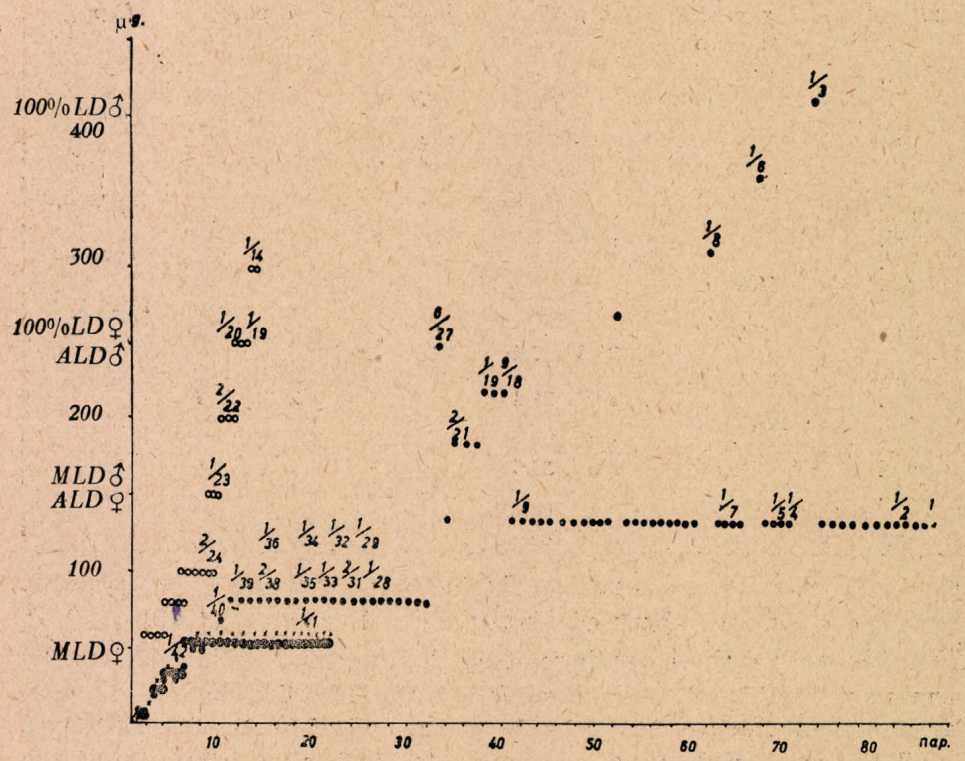

Figure 1. Modes of HT (histamine treatment). x-axis time in days; y-axis dose of H. in μg/100 g. b. w. Each sign (circle, cross or black point) indicates an H injection. In the fractions the denominator is the number of animals injected, the numerator the number of deaths occurred. ooo Group VII.: ••• Group VI.; xxx Group X.; ●●● Group IX.;

solved in dist. water) given subcutaneously (s. c.), and this dose was raised by small additional amounts. (All doses of H are given in μg/100 g. b. w.) Special care was taken to give the s. c. injections only in the upper, the i. p. in the lower abdominal regions. 0.025 ml/100 g. b. w. was the amount of fluid in which the H was injected.

In the Tables we present our results as they were obtained in the different groups and the percentage distribution of mortalities

TABLE II.

The mortalities of normal guinea-pigs characteristic for histamine.

$a = \frac{\text{number of inj. animals}}{\text{number of mortalities}}$ b= Behrens %.

Doses γ/100 g.	Group I. s. c. ♀		Group II. i. p. ♂		Group III. i. p. ♂		Group IV. i. p. ♂		Group V. i. p. ♂	
g.	a	b	a	b	a	b	a	b	a	b
13.	0/10	0								
15.					0/17	0				
25.									0/10	0
39.					0/16	0				
62.					0/16	0				
80.	0/15	0								
115.									3/10	25
143.			0/16	0	0/16	0				
200.									8/10	84.6
220.	6/16	22								
255.									10/10	100
270.	8/16	56								
320.			2/16	7.4	2/16	8 7				
360.	11/14	89.2								
400.			1/4	21.4			2/12	16.7		
410	12/12	100								
500.			1/4	33 3						
600.			2/6	54.5						
640.					7/14	56.2				
700.			3/4	90						
800			16 16	100			12/12	100		

calculated with Behrens's method. In the Figures the "characteristic curves" are shown calculated according to Behrens's (1929) method. With this method the percentage of death for each dose is calculated by forming a fraction whose numerator is formed by the sum of the number of deaths which occurred in the group and of all the deaths caused by all the smaller doses. The denominator is a sum composed of the number of animals in this group and the sum of the differences between the denominator and numerator in the groups of all the higher doses (i. e., the total of the animals which survived larger doses) plus the sum of the deaths which occurred in the groups of lower doses.

EXPERIMENTAL RESULTS.

The "characteristic curve" of guinea pigs for H was determined with the animals of Groups I—V of Table I. The results are shown in Table II. and Figure 2.

From the results of Groups I and V it is clear that the sensitivity or resistance to subcutaneous H amongst the normal guinea pigs is

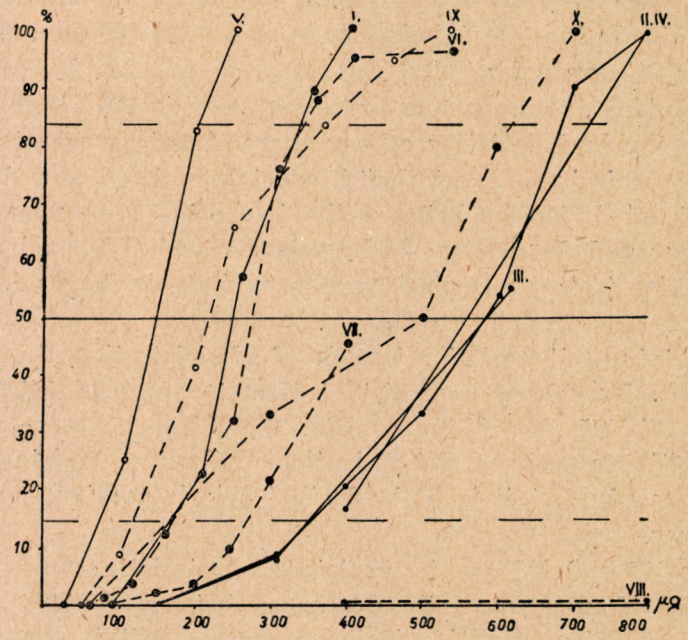

Figure 2. Characteristic curves of different guinea pigs for H. x-axis dose of H in μg/100g b. w., y-axis % of mortality o———o s. c. normal female, Group V; ●———● s. c. normal male, Group I; •———• i. p. normal male, Groups II, III, IV; o·····o HT female, Group IX; ●·····● HT male, Group X; ●·····● HT male, Group VI; ●·····● HT male, Group VII; ●·····● HT male, Group VIII.

distributed normally, the female animals being more sensitive than the male. The ALD for the former found by the integrated curve is 150 μg/100 g. b. w. $\mu = \pm 60$ $\mu_{ALD} = \pm 6$.

The ALD for male animals found by the integrated BEHRENS's curve is 250μ/100 g. b. w., $\mu = \pm 85$, $\mu_{ALD} = 9$. The distance between the ALD male and female is 100, the "k", the significance

of difference = 10. Consequently the difference betwen the male and female ALD cannot be due to sampling but is the consequence of the sex difference.

A comparison of our results with the earlier ones reveals the following relationship: SCHMIDT and STÄHLIN (1929) found that some animals die only after 1000 μg/100 g. b. w., SCHENK (1922) gives 350 μg/100 g. b. w. as the MLD for s. c. H. These are rather high values compared to ours, though the 350 μg 100 g dose falls within our male characteristic curve, not however as MLD. Even in the case of our male animals the 100% lethal dose was only 400 μg/100 g. b. w. and the MLD round about 100 μg/100 g. b. w. The difference (600 μg/100 g.) between SCHMIDT and STÄHLIN's and our 100% lethal dose is 6 times the standard deviation of our characteristic curve at the point of the ALD. This is so great that it must have been caused by some very significant difference between the two sets of experiments. Possibly SCHMIDT and STÄHLIN worked on larger and older animals, gave the H injection to less vascularized s. c. regions, in larger fluid quantities.

To the *intraperitoneal* (i. p.) mode of administration of H the male animal is considerably more resistant than to the s. c. injection. For Group II—III—IV the ALD = 583 μg/100 g. b. w., $\mu = \pm 175$ $\mu_{ALD} = \pm 17$. We made no determination of the i. p. resistance of the female guinea pig, but it is probable that this is greater than that of the s. c. ones.

Groups III and IV are incomplete characteristic curves for i. p. injections. The former covers the dose-range between 0—50%, the latter between 20—100% mortalities. Both curves are within the value of $1\,\mu_{ALD.}$ Indeed the agreement between the results of the three groups at most points of the curve is almost complete. This is the more remarkable because not only the groups differed from one another, but the time intervals between the three experiments was two years.

The distribution of the resistance to intracardiac (i. c.) injection of H amongst the female guinea pigs taken from the paper of Mrs. SMITH-KARÁDY is: ALD = 25 μg/100 g. b. w. $\mu = \pm 9.5, \mu_{ALD} = \pm 1.0$. This value is only about 17% of the s. c. resistance of female guinea pigs.

In describing *the characteristic curves for H of the HT animals,* the peculiarities in obtaining these curves are first to be explained. The experimental results are tabulated in Table III, and Fig. 2.

In groups IX and X the guinea pigs were treated with H as shown in Figure 1. For three weeks the animals in both groups received

an s. c. injection of H twice a day, early in the morning and late in the evening. On the first day the dose was 5 μg/100 g, and by raising the dose by 10 μg/100 g daily, on the 5th day a dose of 55 μg/100 g was arrived at. This dose was maintained for the rest of the three weeks.

At the end of this period the 40 female HT guinea pigs in Group IX were divided into four equal subdivisions. These were given the

TABLE III.

The mortalities of HT guinea-pigs characteristic for histamine.

$a = \frac{\text{number of mortalities}}{\text{number of inj. animals}}$ b= Behrens %

Doses /100 g.	Group VI. s. c. ♂		Group VII. s. c. ♂		Group VIII. s. c. ♂		Group IX. s. c ♀		Group X. s. c. ♂	
	a	b	a	b	a	b	a	b	a	b
55							0 10	0	0 20	0
60			0/24	0						
70	2/80	0.2								
80	13/711	1.1								
100			2 48	0.8						
105							2 10	9		
130	17/281	4.1								
150			1/64	1.4						
170	19/77	12·8								
200			3/61	3.9			5/10	41.1		
250	34/94	32 3	4/54	10						
265							7/10	66.6		
300			1/28	21.5					3/5	33·3
310	35/41	76								
360	37/40	88								
370							8/10	84.5		
400			0/13	45.8	0/13	0				
410	38/39	95								
465							11/3	94 2		
500									2/5	50
540	36/39	97.4								
570							2/2	100		
600									3/4	80
700									5/5	100
800					0/12	0				

doses of 105, 200, 265 and 370 μg/100 g respectively. H injection causes two kinds of death, one within two hours of the injection with the animal showing symptoms of acute H poisoning, the other some time the next day, after the animal had recovered from the effects of acute H poisoning. In the Table only the "acute death" cases are reported. In the four subdivision of group IX altogether 22 animals died an acute, and 5 more a delayed H death. The remaining 13 animals were 3 days later given 465 μg/100 g H s. c. Two guinea pigs survived.

These died after 3 days at the injection of 570 μg/100 g H. During the 3 day intervals the animals received two daily injections of 55 μg/100 g H.

Group X, consisting of 20 HT male guinea-pigs was divided into four equal sub-divisions, receiving 300, 500, 600, 700 μg/100 g H respectively.

The comparison of the characteristic curve of the normal animals (Groups V and I) with that of the HT ones (Groups IX and X) reveals in the case of both sexes the same changes. The most obvious of these is the change in the shape of the curve. In the HT animals the characteristic curve loses its normality and assumes a very marked positive skewness. At the lower doses between 0—40% normal and HT curves run close together.

The MLD is, of course, uncertain in all the groups. It lies probably nearer the higher doses than to the 0 value on the X-axis. Thus the probable MLD for the female HT guinea pig is about 70 μg/100 g. b. w., for the males about the same. As is seen in Fig. 2, these values are about the same in the corresponding normal controls. But the 50% LD is already greater in the HT than in the normal guinea pigs. The 50% LD for HT female animals is 222 μg/100 g. b. w., $\mu = \pm 132$, $\mu_{ALD} = 13$, for the males 500 μg/100 g. b. w., $\mu \pm 215$, $\mu_{ALD} = 22$. The shift towards the larger doses is greatest at the 100% LD this for the HT females being 530 μg/100 g. b. w., for the HT males 700 μg/100 g. b. w.

Owing to this alteration of the frequency of greatly varying sensibility the standard deviation of the ALD of the HT animals is increased. These changes in the characteristic curve mean that HT leaves the lethal sensitivity of a number of animals to H unaltered, whereas in certain other animals considerably decreases it. If HT had caused in each animal a certain increase of resistance the characteristic curve of the HT animals would have retained its normal shape but the entire curve would have been shifted towards the larger doses compared to the normal untreated animals. As is, e. g., the case between the normal female and normal male animals.

In Group VII we compared the effect of a change in the HT on the H resistance of animals. In this Group (see Fig. 1) the animals were given during the first 8 days 3 injections of H. The first day's dose was 4 μg, that of the second day 60 μg/100 g, and so on: 60, 80, 100, 100 μg/100 g on the consecutive days. From the 9th day on the following doses were given: 150 μg,- 200-, 200-, 250-, 300-, 400-, μg/100 g. The difference between this H treatment and the one applied in Groups

IX and X is that here the H doses are being raised rapidly during a short period towards and beyond the lethal values, whereas in Groups IX and X a dose certainly less than the minimum lethal dose was given for three weeks. In Table III column "a" the fractions of the sub-divisions for Group VII are formed as follows: The numerator indicates the number of acute H deaths in this sub-division. The denominator is formed by multiplying the number of the animals with the number of the injections of the dose given to this sub-division. E. g., out of the 24 animals with which we started, 2 died before the dose of 150 μg/100 g was begun. This dose was given once to 22 animals, 1 died, the remaining 21 animals received twice the dose of 150 μg/100 g, and this results in $22 + 42 = 64$ for the denominator.

Comparing the curve of Group VII with that of Groups I and X respectively we see that this curve is incomplete, yet as far as the curve goes it does not agree with curve X. It runs at the small doses parallel to the normal curve but shifted to the higher doses as it approaches the 50% LD it begins to skew away towards the larger doses. We may assume that had the increase of the s. c. injections been continued to the 100% LD the same shaped curve would have been observed as was found in Group X. With reservation therefore we conclude that the repetition during a short period of larger, even lethal, H doses has the same effect on the H resistance of the guinea pig as has a slow HT with less than lethal doses.

Group VIII is formed by those 13 animals which out of the 24 guinea pigs in Group VII survived after HT even the largest (the 100% normal s. c. LD) H dose. These animals were given the normal 20% and the normal 100% i. p. dose, 72 hours being allowed between the two injections. All the animals survived both doses. This is an indication that the animals which have acquired an increased resistance to H given s. c. would probably show an increase against i. p. H injections as well.

In Groups IX and X we saw the effect of a medium long HT with doses well below the MLD, in Group VII that of a short HT with rapidly increasing H doses up to the MLD on the sensitivity to large H doses. In Group VI 40 male guinea pigs were treated somewhat similarly to Groups IX and X, with the difference that after the 10th day when the 55 μg/100 g was attained the dose was slowly increased to 80 μg/100 g and this dose was continued till the 30th day. Then in 3 steps the dose was raised to 250 μg/100 g; i. e., almost the normal 50% LD, each rise having been given for 3 days. This was followed by daily injections of

130 μg/100 g of H until the 86th day. At intervals the surviving animals were tested with large doses of H as indicated in Fig. 1. In column "a" of Table III the fractions of the different doses are formed as in Group VII. On comparing, in Fig. 2, the curve of Groups VI to the normal curve and to the curve of Group X, we see that this curve assumes the same shape as that of Group X. Both curves while running

TABLE IV.

The maximum tolerance of histamine in HT animals succumbing to a smaller dose.

Nr.	Lethal dose μg/100 g.	LD given on how many days?	Survived doses μg/100 g. larger than lethal.
9.	220.	2	250;
71.	130.	22	170×3; 220×3; 250; 270×2; 310;
78.	170	3	170×2; 250;
46.	130	3	170×3; 220×3; 250;
90.	130	27	170×3; 220×3; 250; 310; 360; 250;
29.	220	1	170×3; 220×3; 250; 270; 310;
51.	130	36	360; 410; 450;

fairly close to the normal at the lower doses, show a positive skewness at the higher ones.

The following additional conclusions may be drawn. 1.) The daily repetition of a fairly strong H shock for a long period does not appear to alter (neither diminish nor increase) the resistance once developed. 2.) During the first third of the H treatment the daily dose of 80 μg/100 g was given 21 times, killing on each of the first 14 days some of the remaining animals, thus killing 11 out of 38 animals. Seven of the 40 animals in this group died at a later date of a smaller H dose than had previously been resisted. This is shown in Table IV. These two latter circumstances clearly show that the H resistance of the HT guinea pigs is not a fixed value, but has a periodical oscillation between a minimum and maximum.

From the data of Mrs. Smith-Karády we calculated the characteristic curve of H for intracardiac injection in both normal and HT animals. Here we found that both curves show a normal distribution.

The ALD of the normal females for i. c. given H was found to be 25 μg/100 g, $\mu = \pm 7.5$, $\mu_{ALD} = \pm 0.8$. The same values for the HT animals are: ALD 55 μg/100 g, $\mu = \pm 7.5$, $\mu_{ALD} = \pm 0.8$. The significance of difference for the difference between the normal and the HT ALD is, $k = 26$.

DISCUSSION.

The experiments described answer as follows the question whether histamine desensitization exists or not: It appears certain that after HT the resistance of each and all of the pre-treated animals does not increase with an individually varying but definite value. This clearly follows from the circumstance that the mortality curve loses its normal shape and is not shifted towards the larger doses with a distance at least twice that of the standard deviation. The most obvious effect of the HT in both sexes is the positive skewness of the curve. This means that after HT a certain number of the treated animals acquires a resistance considerably greater than that of the normal 100% mortality value, and that the value of the lethal doses from 60% upwards is also increased. Is this caused by the HT, or is it merely a sampling effect? The distance between the ALD for normal males (G. I. 250 μg) and the 100% LD for HT males (G. X = 700 μg) = 450. The μ for the normal males is $\pm$ 85. By dividing the 450 by 85, we find that the 100% LD of the HT animals lies 5.3 μ distant from the normal ALD. This means that amongst 10,000.000 normal male guinea pigs one will occur which is able to resist the 100% lethal dose (700 μ) of the HT animals. Whereas after HT 5 out of 100 treated animals will be enabled to resist this or perhaps even higher doses.

In the case of the female animals the normal ALD (G. V.) is 150, the 100% LD of the HT females (G. IX.) is 530. The μ for the normal female cases is $\pm$ 60. The difference between the 100% LD of the HT females and the ALD of the normal females divided by the normal $\mu = \frac{530 - 150}{60} = 6.3$ normal μ. This means that it will take well over 10,000.000 normal females to find one with the resistance of the 100% HT lethal dose.

Unlike our results, Mrs. SMITH-KARÁDY's point to a general and even increase both in the s. c. and i. c. testing of the resistance after HT. Her normal subcutaneous results also differ from ours. This difference is possibly caused — as she kindly informed us personally — by the fact that her guinea pig population was not definitely homogen-

eous as regards virginity. Since ours was from this point of view strictly homogeneous — and showed a smaller standard deviation — we feel entitled to attribute the difference to this inhomogeneity and not to that between the time or place (her experiments were carried out some 6 years before ours; she worked in North Dakota, and we in Hungary).

As regards the effect of the time-interval between two series of experiments, it is instructive that we have several series of experiments differing from one another in one to two years (Groups I and VII, Groups II and III, yet their curves fell well within one μ of the ALD. Besides being inhomogeneous Mrs. SMITH-KARÁDY's data are also incomplete. We are therefore inclined to attribute the differences between her own normal and HT results and ours to the circumstance that her inhomogeneous population had a great standard deviation. This means that in the lower doses there is no difference between her normal and her own normal and HT results and ours to the circumstance that her observed the same agreement. She failed to observe in the course of her work the possible positive skewness of her curve because these high doses had not been tested.

Though in the case of the s. c. results the differences between Mrs. SMITH-KARÁDY's and our own may in this way be understood, her i. c. results definitely support the conclusion of an even increase of resistance. There are two possible explanations for this difference. One is that the mode of action of a s. c. given H is different from that of an i. c., and that the HT alters just this differing link in the chain of physiological processes. The other is that the HT i. c. curve is in the large dose range actually based only on two groups.

A few words must be said about the observations proving that the sensitivity of one and the same animal to one and the same drug is not a fixed value, but varies from time to time. It has long been claimed by several pharmacologists that the variability of the lethal doses is due to the characteristic coincidence of the variations of the sensitivity of one and the same animal (GADDUM l. c. 1933; MORELL and CHAPMAN, 1933). Our experiments furnish additional evidence in favour of this theory. It must be pointed out that in no cases out of the 7 deaths from a smaller dose did death take place on a day following the day of a large dose. It is therefore impossible that this relatively decreased resistance was caused by this preceding larger dose. The recurrence of the same sensitivity on a later date as described above is, of course, the consequence of this same oscillation of the resistance.

From this it is clear that when the characteristic curve of a population of animals is tested, the observed frequencies are not due to the sampling of animals of different sensitivity with a fixed value, but to the coincidence of certain sensitivities oscillating within limits. How great can this oscillation of the sensitivity in the same animal be?

From Table IV it was seen that it is possible that one and the same animal may have a sensitivity of 130 μg/100 g or less, and may exhibit on a previous day a sensitivity enabling it to withstand 450 μg/100 g. That is to say, it is possible that in one and the same animal the sensitivity from day to day may vary between the lower and higher asymptote of the drug. The standard deviation of a sensitivity means therefore the range within which $\frac{2}{3}$ of the variations of the periodical oscillation of the sensitivities of one and the same animal will fall.

Accordingly, the distance of any given sensitivity from the ALD expressed in standard deviation would mean the frequency with which this sensitivity will occur in any animal out of the total of its sensitivity's variations. E. g., a sensitivity 3.09 distant from the μ_{ALD} does not mean that amongst 500 animals one will occur with this sensitivity, but that any of the animals have periodical oscillations of sensitivity round the ALD and that out of 500 oscillations, one will occur with this sensitivity.

The other possibility is a combination of the standard individual value of resistance with smaller oscillations around this value. Which of these two possibilities is the one actually existing, and whether there are drugs towards which one, and others towards which the other, is manifested are questions awaiting further experiments for their elucidation.

The relationship between experiments and the observations on the alleged H desensitization against surgical shock in man remains to be discussed. The two sets of observations differ: in the mode of the HT in the physiological properties of the two observed organisms (man versus guinea pigs), and finally in the mode of testing (lethal dose of H versus surgical shock) the change brought about by the HT.

The mode of the HT in the case of man consists in giving for 8—10 days 0.5—1 mgm-g H twice a day, whereas to guinea pigs 7—13 times larger doses are given. Further, man with his 70 years' average life-span is treated for about a week, the guinea pig with its life-span 1/10th as short is treated for 2—3 weeks. Significant as these differences are, the differences between the mode of action of a lethal dose of H on the guinea pig and that of surgical shock in man are still

greater. The most important of these differences is that the accumulating experimental evidence, though favouring the humoral origin of the *Bayliss-Cannon* shock hypothesis, appears to disprove the idea of the shock being essentially a histamine poisoning. This circumstance naturally excludes the possibility of using directly the results of the HT in guinea pigs as an argument in favour of the existence of a histamine desensitization against surgical shock in man. It must, however, be emphasized that though our experiments cannot be used to support this hypothesis, they do not argue against it either. The question of the existence of histamine desensitization in man against surgical shock, difficult as it is, can be decided only on well controlled and statistically sound human material.

SUMMARY.

1. In normal guinea pigs of both sexes and in those which underwent a pretreatment of repeated injections with small doses of histamine (HT animals), the "characteristic curve" for s. c. histamine injections was determined.

2. The ALD for H in normal female guinea pigs is 150 μg/100 g. b. w. $\mu_{ALD} = \pm 6$, same for normal males 250 μg/100 g. b. w. $\mu_{ALD} = \pm 9$. The female guinea pig is 66% more sensitive to this drug than the male.

3. The „characteristic curve" of HT guinea pigs loses its normal shape and assumes a very pronounced positive skewness. Part of the animals therefore develop an increased resistance in consequence of the HT while the sensitivity of another part of the animals remains unaltered.

4. Experimental evidence is presented proving, in the HT animals, the oscillatory nature of the resistance of one and the same guinea pig towards H. It is suggested that the characteristic curve is formed rather by the regular coincidence of certain sensitivities oscillating within limits than by distribution of animals with standard but individually varying sensitivity.

5. Whether HT is carried out with doses considerably below the MLD for a medium time (3 weeks) or with doses rapidly (in one week) reaching the lethal dose range does not seem to alter the result of the HT. The maintenance of H shocks with 40% LD for a long time neither increases nor diminishes the acquired resistance of the animals.

6. The authors emphasize that the results of their experiments are

not to be used as arguments in favour of the existence of histamine desensitization in man against surgical shock.

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REFERENCES.

BARLOW, O. W., and HOMBURGER, E. (1941). *Fortschr. d. Therap.* **13.** 568.
BEHRENS, (1929). *Arch. exp. Path. Pharmacol.* **140.** 237.
BRETSCHGER (1936). *Dtsch. Z. Chir.* **246.** 377.
CLARK, W. G., MACKAY, E. M. (1939). *Proc. Am. Phys. Soc.* **49.**
FARMER, L. (1939a). *J. Immunol.* **36.** 37.
FARMER, L. (1939b). *J. Immunol.* **37.** 321.
GADDUM, J. H. (1933). *Med. Res. Coun. Spec. Rep. No.* **183.**
HORTON, B. T., MACLEAN, A. R., and CRAIG, W. (1929). *Proc. Staff Mayo Clinic* **11.** 257. (April 26.) (cit. after 9).
KARÁDY S. (1938). *Amer. J. Physiol.* **123.** 194.
KARÁDY, I. and BENTSÁTH A. (1935). *Zschr. f. klin. Med.* **128.** 640.
KARÁDY, St. (1936). *Arch. f. exp. Path. u. Pharm.* **180.** 283.
KARÁDY, St., and BENTSÁTH, A. (1936). *Ztschr. exp. Med.* **100.** 48.
KOKAS F., SARKADY L., and WENT, I. (1938). *Magy. Orv. Arch.* **39.** 408.
MACKAY, E. M., CLARK, W. G. (1938). *Proc. Soc. Exp. Biol. a. Med.* **39.** 56.
MILKO, V. (1936). *Sitzung f. ung. Ges. f. inn. Med.* 2. VI.
MORELL, C. A. and CHAPMAN, C. W. (1933). *J. Pharm. exp. Ther.* **48.** 381.
RUSZNYÁK, I., KARÁDY, I., SZABÓ, D. (1934). *Orvosi Hetilap* **14.**
RUSZNYÁK, ST., KARÁDY, ST., SZABÓ, D. (1936). *Arch. f. klin. Chir.* **187.** 279.
SMITH-KARÁDY E. (1940). *M. S.*
SCHENK, P. (1922). *Arch. f. exp. Path.* **92.** 34.
SCHMIDT, G. W. and STÄHLIN, A. (1929). *Ztschr. f. Immun. forsch.* **60.** 222.
TREVAN, J. W. (1927). *Proc. Roy. Soc. B.* **101.** 483.
TREVAN, J. W. and BOOCK, E. *Doc. C. H.* **398** *of the Health Org. of League of Nations.*

THE MECHANISM OF THE INCREASE IN RESISTANCE AGAINST LETHAL DOSES OF HISTAMINE DEVELOPED BY PREVIOUS INJECTIONS OF SMALL HISTAMINE DOSES.

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(With 1 Figure and 4 Tables in the text.)

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In a previous paper (Beznák, Kovách, Gáspár-Rády, 1946), we have shown that repeated injections of small doses of histamine (HT = histamine treatment) cause in certain guinea pigs an increase of resistance against the normal 100% lethal dose. Such animals with increased resistance succumb only to a dose which is 3—6 standard deviation far from the normal ALD. As described in the paper cited, the HT does not increase the resistance of all animals of the treated population evenly, it leaves the resistance of many of them unaltered.

In the present paper we describe our experiments dealing with the physiological mechanism of the increase in this resistance.

Previous works: There are few papers describing direct experiments bearing on the physiological mechanism of H-resistance. It was claimed by Karády (1935) that the fall in the arterial blood pressure in H-desensitized cats is smaller than in normal ones. Kokas, Sarkady and Went (1938) could not confirm this result. Control experiments carried out by one of us in collaboration with Csáky (Csáky & Kovách, 1947) proved that neither of the two experimental procedures was satisfactory. Experiments in which the complete concentration-action curves were compared in the same cats before and after HT proved clearly that HT does not alter the arterial blood pressure response to H in the cat.

HT was found in rabbits to diminish the decrease of the blood volume after a large dose of H and after traumatic shock. (Karády, 1938.) This same was found earlier in dogs as far as the traumatic shock is concerned (Rusznyák, Karády & Szabó, 1936).

A more or less complete theory considers HT as a special case of the "alarm reaction of SELYE" (KARÁDY, SELYE & BROWNE, 1938 a, b). According to the alarm reaction theory, the first application of any non-specific, fairly strong stimulus (injection of formaldehyde, application of cold, physical exercise) provokes from the organism the first phase of the alarm reaction. By this the animal becomes more resistant to the second application of any such non-specific alarm stimulus. In the development of this resistance the hyperfunction of the suprarenal cortex plays a decisive role (SELYE, 1937). Independent of the question of whether HT is a special case of the alarm reaction postulated by SELYE's theory or not, it is a fact that it causes in the rat suprarenal hypertrophy (MACKAY and CLARK, 1938). The circumstance that non-specific stimuli of varied kinds may be neutralized by the same single agent (the suprarenals) is explained hypothetically by supposing that all of them release histamine, and it is this hypothetical common physiological denominator which the hyperfunctioning suprarenal counteracts. (SELYE, 1937). It is indeed well known that the H-resistance is decreased in the epinephrectomized animal and is restored by desoxycorticosteronacetate. (NOBLE & COLLIP, 1941). In the blood of adrenalectomized animals the H-content is 21—230% elevated and suprarenal cortex-extract restores it to almost the normal level. It is significant that in fake operations which may be regarded as an alarm stimulus, the H-content decreases (WILSON, 1941). The mode of action of the suprarenals, both in the alarm reaction and in the H-desensitization, is unknown.

A few words must be said about the mechanism of the acute increase of resistance to H caused by rapidly repeated injections of H (H-tachyphylaxis). It was proved by EICHLER & KILLIAN (1931) and by EICHLER & MÜGGE (1931) that in this resistance the developing incompensated acidaemia plays a role. Acidaemia relaxes the spasms of the bronchioles caused by H-injections. With increasing doses of H the acidaemia is also increased but not the broncho-constriction. It may be mentioned in this connection that M. BEZNÁK, KORÉNYI & HAJDU (1942) produced evidence that the suprarenal hypertrophy and hyperfunction caused by exercise is brought about by the increase of the concentration in the blood of muscle metabolites, primarily of acids.

In planning the course of our search for the mechanism of the increase of resistance, we proceeded as follows: Guinea pigs made resistant by HT are killed by a dose considerably higher than the 100% normal LD. The symptoms of the mechanism of death do not differ in the normal and in the resistant, HT guinea pigs. The mechanism of death caused by H, as proved by the experiments of DALE and LAIDLAW (1910—11), consists, in the guinea pig, of suffocation due to a diminished output of O_2 blood by the left ventricle. This is the consequence of a very powerful broncho-constriction and diminution of the blood-flow through the lungs. This latter in its turn is caused partly by the constriction of the pulmonary arterioles, partly by the failure of the heart ventricle which is conditioned by the inadequate supply of oxygenated blood. If therefore a 100% LD of H does not

kill a resistant HT animal, this must be due to either a better output of O_2 blood or to an increased resistance to lack of O_2 blood by the tissues. O_2 determinations carried out at the peak of H-shock caused by a 100% LD of H in the arterial blood of normal, non-resistant- and resistant- HT guinea pigs, decided in favour of the first possibility: the lungs of the resistant HT animals oxygenate far better than do the normal or the non-resistant guinea pigs.

Theoretically there are two possibilities which might bring about this change. HT either made the broncho- and vaso- constrictors in the lungs less responsive to the same level of H in the blood, or reduced the concentration of H in the blood following the s. c. injection of the same dose of H. Determinations of the H content of the blood showed a small and irregular tendency towards a reduced H content in the HT animals. These estimations showed, therefore, that the better oxygenation is the consequence of the decreased response of the broncho- and vaso-constrictors to the same blood level of H. Estimations of the rate of adsorption from the peritoneal cavity and estimations of the rate of disappearance of i. c. injected H have also been made, to find out whether this small and irregular decrease was not due to an alteration of the dynamic equilibrium between these two factors.

Since EICHLER *et al.* showed that incompensated acidaemia causes the bronchoconstrictors to relax, we carried out comparative lactic acid and CO_2 determinations in the blood.

METHODS.

In the comparison of the blood O_2 and CO_2 content, 37 male guinea pigs of 611 g $\mu \pm 34$ g were used. The experiment were made between 17. 7. 1944 and 31. 12. 1944. In 8 of them we determined the O_2 and CO_2 content of the normal carotid blood of the guinea pigs (Group. I.). All animals survived this operation. In 5 normal guinea pigs we suceeded in taking presumably premortal blood from the carotid at the peak of the H-poisoning (Group II.). A few minutes later all animals died. 24 animals had been pre-treated with H as described below. At the conclusion of the HT they were given the normal 100% LD (410 μg/100 g). When menacingly severe symptoms occurred blood was drawn from the carotid. The animals which died make up the non-resistant HT Group III.; those which survived form the resistant HT. Group IV; If such symptoms did not occur we waited

for 30 minutes, and carried out the O_2 and CO_2 determinations in the carotid blood. These animals are also registered in the resistant HT Group IV.

The withdrawal of blood was carried out as follows: The animal was tied to the operating table. In local novocaine anaesthesia the external jugular vein was cannulated, 3 mg/100 g of Liquoid La Roche was slowly injected to prevent clotting. Canulla was introduced into the carotid on the same side. The 100% s. c. normal lethal dose, 410 μg/100 g b. w. in 0.1 ml. of tyrode, was injected into the subcutis of the upper, middle abdominal region. The time of the withdrawal of blood is described above. Between 2—4 ml of blood were withdrawn. In 1 ml the O_2 and CO_2 contents were determined, by VAN SLYKE's manometric method (1927). In 1 ml. of blood the H content was determined by CODE's method (1937) as modified by EICHLER and SPEDA (1940).

The HT was carried out as follows: on the 1st and 2nd days 0.01, on the 3rd and 4th 0.02, from the 5th day onwards for 14 days 0.03 mg/100 g. b. w. H was given twice a day 0.1 ml/100 g. b. w. of tyrode s. c. into the upper middle abdominal region.

The rate of adsorption from the peritoneal cavity was determined in 12 normal and 12 desensitized animals. These were given 650 μg/100 g b. w. H base i. p. (60% of the 100% normal i. p. LD into the lower middle part of the abdominal region. Half an hour later the animals were decapitated and bled out, the abdomen laid open, its content allowed to drain thoroughly and the abdominal cavity washed 3 times with saline. These determinations were carried out between July 22nd, 1943 and September 12th of the same year. The H determinations were carried out with BARSOUM & GADDUM's method as modified by CODE (1937). The HT of these animals began with 10μg on the first and 2nd day, then the dose was raised as follows: 3rd and 4th days, 20; 5th and 6th, 30; 7th 40, 8th and 9th days 50, 10th day 60, 11th day 70, 12th 80, 13th 90, 14th 100 μg. Each dose was given once a day. Each dose in μ gs/100 g. b. w.

The determinations of lactic acid in the blood were carried out with the method of LAURENSEN and WAHLLÄNDER (1938).

In a few cases we determined the blood flow from the carotid using the following method: The guinea pig was given 3 mg/100 g. b. w. Liquoid La Roche in 0.2 ml. of tyrode solution in the external jugular vein. The left carotid was cannulated in Novocaine anaesthesia, tests of the blood flow were taken at intervals by releasing the Spencer-

Wells in the carotid and then counting with a stop-watch the time intervals of drops of blood.

EXPERIMENTAL RESULTS.

In Table I. we present the results of determinations of O_2 and CO_2 in the carotid blood.

TABLE I.

The O_2 and CO_2 content of the carotid blood in normal and HT guinea-pigs.

No. of animals columns:				Normal values		H-killed normal animals		H-killed HT animals-non-resistant		H survived HT resistant animals	
1	3	5	7	1 O_2 %	2 CO_2 %	3 O_2 %	4 CO_2 %	5 O_2 %	6 CO_2 %	7 O_2 %	8 CO_2 %
XI	16	1	26	19.5	28.8	0 36	65.6	4.55	55.2	4.88	49.4
IV	12	2	22	15.7	27.2	0.18	48.8	3.35	57.2	10.10	47.1
XV	13	24	19	12 9	36.1	0.41	63.1	0.98	47.5	8.62	43.5
VIII	14	28	16	17.9	28.1	4.26	50.6	2.45	55.2	9.66	40.2
XVIII	15	17	41	12.7	29.4	0.51	63.1	2.95	51.0	15.82	38.7
XVI		11	3	10.6	35.0			0.96	49.7	6.17	35.1
XIV		40	23	15 8	28.3			1.28	65.1	13 92	32.2
XII		9	11	19.7	38.7			1.46	44.2	11.50	31.9
		12	10					6.54	40.0	16.20	42.2
		40	14					4.02	34.8	13 60	31.4
		15						7.14	33.2		
		8						4.60	45.6		
		18						3.80	36 8		
		16						1.81	35.6		
No. of cases				8	8	5	5	14	14	10	10
$M = \frac{\Sigma x}{n}$				15.63	31.50	1.14	59.00	3.28	47.70	11.14	32.29
$\mu = \frac{\Sigma dx}{n} . 1{\cdot}25331$				3.35	4.83	1.57	8.96	2.00	9.72	3. 87	7.14
$\mu_x = \frac{\mu}{\sqrt{n}}$				0.94	1.71	0.74	4.02	0. 53	2.59	1.38	4.61

As is seen from Table I., in the normal animal death ensues when the O_2 content of the carotid blood falls from 15. 63 vol% to 1.14 vol%; i. e., when the blood contains practically no O_2 (Table I. column 3.).

Attention is to be called here to the circumstance that in this average in 4 out of the 5 cases the O_2 content did not reach 0, 6%, but in one of the 5 cases it attained 4.26%.

When HT guinea pigs died in consequence of s. c. H injection, the average O_2, content is 3.28 vol%. The significance of the difference (k) between these two values is 5.03. It is therefore certain that the HT animal which did not become more resistant to the lethal effect of H is more sensitive to the O_2 lack than the normal. The most striking difference is to be seen in the group of the animals which developed resistance to the lethal dose of s. c. given H. Here the average O_2 content is 11.14%, i. e., 70% of the normal 15.63% value. We can safely conclude that the HT animal when it becomes more resistant does not succumb to the normal s. c. 100% LD of H, because some change allows a better oxygenation in the lungs than that which takes place either in the normal or in the non-resistant HT animals. It is to be remembered that the HT animal, when its resistance has not been increased, dies at a higher O_2 content of the arterial blood than does the normal animal, but even this decreased resistance to the lack of O_2 is well overcompensated by the great amount of O_2 in the blood leaving the lungs.

On the passage of blood through the pulmonary circulation in the normal and HT animals killed by H we made the following observation: When blood is collected from the carotid of the normal guinea pig, the time between 2 drops is measured in seconds. The time required between 2 drops of blood in the normal animal at the peak of an H poisoning is 10 times as long. About the same time is necessary in the non-resistant HT animals, while in the resistant HT guinea pigs the time approaches the value obtained in the normal non-H-poisoned animal. It has been proved by FIELD and DRINKER (1930) that histamine causes in the guinea pig a contraction of the pulmonary arterioles. Since the output of the left ventricle increases in the resistant HT animals, we suppose that in these cases H does not cause the same constriction it does in the normal or non-resistant HT animals.

This increased oxygenation in the resistant HT animals may be due to an unaltered broncho-constrictor reaction to lower H level in the blood, or to a tardier, broncho-constriction to the same H level in the blood reaching the lung.

A lower blood level of H may be the consequence of a slowing down of adsorption. As Table II shows, the adsorption from the peritoneal cavity is almost unaltered in the HT animals, though there is a tendency in them to slower absorption.

It is clear from these values that there is very little slowing down of the adsorption in the HT animals. Attention must be drawn to the

circumstance that the standard deviation of the mean value of adsorption in the HT animals is not greater but smaller than that of the normal controls. This is significant because only part of the HT

TABLE II.

The absorption of H from the abdominal cavity in normal and HT guinea-pigs following the i. p. injection of 0.65 mg/100 g. b w. of H.

No.	Total injected µg	Remained in the peritonial cavity µg	Absorbed µg	dx	Remained %	Absorbed %	dx
88	2 230	234	1.996	117	10.4	89 6	5.6
70	3.000	600	2.400	287	20.0	80 0	4 0
75	2 300	240	2 060	53	10.4	89.6	5 6
77	2 500	440	2.060	53	17.6	82 4	1 6
82	2 660	272	2.388	275	10.2	89 8	5.8
73	2 030	368	1.662	451	18.1	81.9	2.1
83	2.600	448	2 152	39	17.2	82 8	1.2
74	2.350	560	1.790	323	24.2	75 8	8.2
81	2 350	324	2.026	87	13 7	86 3	2 3
71	2 600	264	2 336	223	10.1	89 9	5 9
79	2.800	368	2 432	319	13 1	86 9	2.9
95	2 850	800	2 050	63	27 0	73 0	11.0
			M_1=2,113	191		M_I=84	4 7
	Norma	control animals					
19	2.500	164	2 336	128	6.6	95.4	6.1
22	3.100	216	2 884	676	7.0	92.7	3.7
28	2.230	171	2 059	149	7 6	92.4	3 1
26	2.850	220	2.630	422	9.4	90 6	1 3
27	2.730	360	2.370	162	13.2	86 8	2.5
16	2.030	222	1 808	400	10.9	89.1	0.2
21	2.340	186	2 154	54	7.9	92 1	2.8
22	2.340	217.6	2.122.4	86	9.2	90.8	1.5
17	2.340	108.5	2 108 5	23	4.6	95 4	6.1
18	2.600	240	2.360	152	9.2	90.8	1.5
29	2.210	235 2	1 974 2	232	10 6	89.4	0 1
23	2.350	780	1 570	638	33.6	33.4	23.1
			H_2=2 208	260		M_{II}=89;3	4 33
	M_1-M_2=95 µg				M_I-M_{II}=5.3%		
	μ_1=239.1 ; μx_1=70.3				μ_I =5.84 ; μx_I =1 71		
	μ_2=325.1 ; μx_2=95.2				μ_{II}=5.43 ; μx_{II}=1.59		
	k (M_1 M_2)= 0.599				k (M_I M_{II})=2.26		

animals develope resistance — as shown in the previous paper. Others retain their normal sensitivity. It must be emphasized at this juncture that these 12 animals were those which survived a very energetic s. c. HT at the end of which the last dose was the 100% s. c. LD. They

were therefore more or less resistant. If this increase in the resistance in a few cases was due to a retarded adsorption, this latter should show in a few cases an outstanding slowing down, and this in turn should cause an increased standard deviation. However there were no animals in which the velocity of adsorption was outstandingly slowed down.

These are results of adsorption from the peritoneal cavity, whereas we made our O_2 and CO_2 determination in animals which had been poisoned by s. c. injections. Thus we have to suppose that the changes which took place during the HT in the capillary walls, in the venous circulation, in the colloid osmotic pressure and in other factors influenc-

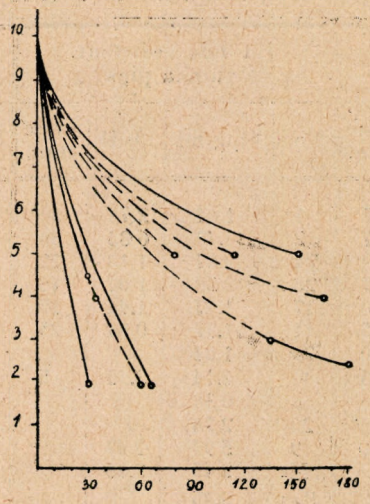

Fig. 1. The H content of the blood-plasma in normal and in HT animals after the injection of 10 μg/ml plasma H i. c. x-axis time in seconds after the i. c. injection of H, y-axis H content μg/ml blood plasma.

ing adsorption are the same, both in the peritoneal and subcutaneous circulation. If this is true we can expect that the change in the rate of adsorption is also the same in both cases; i. e., if there is a slowing down of H adsorption the same change takes place also in the sub-cutis.

The results of the experiments on the rate of disappearance of i. c. injected H from the arterial blood are shown in Figure 1. In this Figure the abscissa is the time in secs. elapsed between the i. c. injection of 10 μg/ml blood plasma of H and the withdrawal of blood sample from the left heart. The ordinate is the concentration of H in

μg/ml of blood plasma. As is seen in this Figure, the concentration of H decreases in the arterial blood very rapidly; the value of 2—5 μg/ml is reached within 30—165 secs. The values of normal and HT animals agree very well. As far as these 2x4 determinations go, there is no difference between the rate of disappearance of H from the blood between the normal and HT animals. The rate of adsorption being irregular and slightly reduced, the rate of disappearance from the blood remaining unaltered, one expects in the HT animals an irregular and slight reduction in the level of the blood H after s. c. injection.

TABLE III.

The H content of blood plasma of normal and HT guinea-pigs at the top of the symptoms of H poisoning following the s. c. injection of the 100 % L D

Normal guinea pigs				HT non resistent guinea-pigs				HT resistent guinea-pigs			
No.	Hμg/ml	O_2/H	Time min.	No.	Hμg/ml	O_2/H	Time min.	No.	Hμg/ml	O_2/H	Time min.
1.	5.92		7	1	1.64	2.78	30	26	2 62	8.94	30
2.	3.47		27	2	0.49	6.83	30	22	3.68	2.88	30
3.	0 88		33	24	0.87	1.10	22	19	1.48	5.83	30
4	2 02		17	28	2.28	1.07	15	16	3.28	2.94	30
5.	2 32		20	17	1.97	1.44	15	4	1.15	13.60	30
6.	4.59	0 06	15	11	1.97	0.84	25	3	1.24	4 96	15
11.	5 28	0 05	30	20	1.64			18	4.96		
12.	8 58	0,02	35	40	1.14	3.53	5	23	5.02	2.78	30
13.	13 12	1.57	20	6	0.38			38	12.10		
14.	2 71	0.27	25	25	2 26						
15.	2.46	0.40	10	9	5.92	2.47					
M_1	4.65	0.49		M_2	2,45	2.46		M_3	3.91		
μx_1	0.98			μx_2	0.45			μx_3	0.94		
k_{1-2}	2.00			k_{3-2}	1.39			k_{1-3}	0.54		

Finally, in Table III. we present our H estimation in the blood of normal and HT animals.

The calculation of the significance of difference between the normal lethal level of H in the arterial blood and the level of H in the blood of those HT animals which resisted the 100% s. c. LD shows that these 2 values agree very well (k_1—k_3=0.5). The slight tendency towards a decreased H concentration is in accordance with the slight tendency to a slowing down in the adsorption of H into the blood. These experiments provide clear-cut evidence in favour of the mechanism in which the resistance towards this s. c. LD of H is due to better oxygenation of the blood in the lungs in the presence of the LD of H. This con-

clusion becomes even more evident if we compare the vol% of $O_2/\mu g$ H values. Here we see this value is 0.4 for the normal, whereas for the resistant HT it is 6. The oxygenation of the blood in the resistant HT animal per unit weight of blood H is therefore 15 times greater than in the normal one. It is evident that even in those cases where the slowing down of the adsorption of H into the blood took place and resulted in a reduced blood H this plays only an inferior role in the mechanism of resistance. Considering the mechanism of death and H content in the HT non-resistant animals, we saw that these animals oxygenate much better than do the normal ones. (3.27 vol% O_2 as

TABLE IV.

The lactic acid content of the blood of normal and HT animals during the symptoms of H poisoning

	Normal	HT
No. of cases (x)	10	8
$M = \frac{\Sigma x}{n}$	32 mg%	46 mg%
$\mu = \frac{\Sigma d x}{n} \cdot 1{\cdot}25331$	9·6	11·4
$\mu_x = \frac{\mu}{\sqrt{n}}$	3·	4·
$k = \frac{M_1 - M_2}{\sqrt{u_1^2 + u_2^2}}$	2·8	

against 1.14%). Their blood H with its average of 2.45 μg/ml. is the smallest among the 3 groups, but the significance of difference is very low in both combinations. The quotient O_2 vol%/H is 2.47, the non-resistant HT animal oxygenates 6 times better than the normal ones. But it is obviously more sensitive to the lack of oxygen, since it dies at the average value of 3.28%. The normal dies as a rule at 0.4%, and only occasionally at 4.26%. Though the lung oxygenates better, it does not do so at a sufficiently good rate to raise the O_2 content of the blood high enough to satisfy the increased O_2 hunger of the tissues. This is the case only in the resistant HT animal.

Passing to the lactic acid determination, we see in Table IV. that the blood of the HT animals contains more lactic acid than does the normal one.

As regards the CO_2 content of the blood, we see in Table I. that it is elevated as compared to the normal value in all H-poisoned animals and is normal in the resistant HT animals. The acidaemia is clearly smallest in the group where the bronchoconstrictors relax the most — in spite of the presence of H; consequently the relaxation cannot be due to acidaemia.

Some clue to the nature of the changes brought about by the HT may be gained from the post mortem examinations. The results recorded here have been obtained partly on animals whose details, such as mode of HT, lethal dose, length of survival, etc. have been recorded in our previous paper (Beznák, Kovách, Gáspár-Rády 1947).

In the 3 kinds of animals: normal, non-resistant HT and resistant HT all killed by H, 2 types of situs have been observed.

The following description gives a picture of the unmixed clear-cut types. Combinations of the different symptoms have occurred.

a.) First Type: Seen in the acute histamine poisoning of the normal animal.

In the heart the auricles, especially the right one, were filled to the maximum with blood; out of the ventricles the left one was found in systole containing but little residual blood. The right ventricle, on the other hand, was dilated to its utmost capacity with much blood, imitating diastole. The coronary venae were full of strongly reduced blood. *The lungs* were non-collapsible, dilated to the maximum, fairly congested, slightly edematous. (Auer-Lewis). *The abdominal organs, the liver,* but for varying degrees of congestion (dilated venae centrales), appeared normal. Turbidity or fatty degeneration of the parenchym was not perceived. *The intestines* showed all signs of congestion with occasional small bleedings. Apart from very little pronounced venous congestion we found no change in *the kidneys.* The *suprarenal cortex* was obviously hyperaemic with arterial blood. It is to be noted that the symptoms are always equally severe whether death was caused by a small or by a large dose.

b.) Second Type: Seen in animals whose resistance had not been increased by the HT.

Whereas the normal animals die within 20 minutes after the s. c. or i. p. H injection, out of the 13 non-resistant animals 9 (70%) died only several hours after the injection. At autopsy the picture of *the heart* appeared to be similar to that seen in the first type animals, though perhaps turbidity could be seen. In *the lungs* infarcts have been seen regularly; their size varied from the size of a bean to such an

extent that one or several of the lobes became indistinguishable from the pneumonia "hepatisatio rubra" stage. Those parts of the lungs which had not been affected showed the usual AUER-LEWIS type. Signs of severe chronic congestion were present in *the liver* in all cases; turbidity and fatty degeneration were regular. *The intestines* were not so full of blood as in Type I, yet the signs of chronic congestion were apparent. They were empty and atonic.

The distribution of the two types within the HT animals, Groups I—VI (first paper) was as follows: The second type was mostly seen in Group IV. Out of the 38 autopsied animals in this group 13 died from doses 80—130 μg/100 g during the first part of the HT. In 12 of the 13 (92%) the typical severe symptoms in the lungs were seen. From the 25 HT animals which died later from larger doses in only 14 cases (56%) were infarcts observed. It is significant that they were never as extensive as in the case of animals which died from smaller doses. Severe liver changes were seen in 46% of the sensitive HT and 12% of the other animals.

Between the situses of the *normal* and the *resistant HT animals* (i. e., those killed by larger than the normal 100% lethal dose) killed with H, only one marked difference was found at autopsy. The general picture in these was that of an acute H-poisoning (Type I) but in the HT animals on a background of *chronic venous congestion,* without the severe changes of the parenchyms of the organs.

If we compare the results of the autopsies, it is clear that in those non-resistant HT animals which died from small H doses the symptoms of a chronic venous congestion, and especially its consequences in the parenchym of the heart, lungs and liver were much more pronounced than in the case of either the normal or the resistant-phase animals. Besides this severe chronic venous congestion, the almost regular (12 out of 13) appearance of extensive pulmonal infarcts distinguished non-resistant animals from both the normal and the resistant ones.

DISCUSSION.

The discussions of the O_2 content of the arterial blood of the normal, non-resistant HT and resistant HT guinea pigs showed that in the normal animal at the height of a mortal H-shock provoked by a 100% LD, the O_2 drops from the physiological level of 16%, to 1%; in the non-resistant HT animal to 3%. In the resistant HT

guinea pig during or after the shock provoked by the 100% LD — which, however, these animals survived — the O_2 content sinks only to 11%. These figures warrant the conclusion that a link in the chain of life-processes, which enable some HT animals to resist the normally 100% LD of H, is that HT brings about some change in the organism of the guinea pig in consequence of which better oxygenation takes place in the lungs. Since following a s. c. injection of H in the normal and in the non-resistant HT and in the resistant HT animals, the H level in the blood at the height of the shock does not show a significant difference, yet in the normal practically no, in the non-resistant HT hardly any, and in the resistant HT animals fairly good oxygenation takes place, it follows that the change brought about by the HT consists in enabling the lungs to oxygenate better in spite of the presence of such a concentration of H in the blood as makes this oxygenation otherwise impossible. (The slight and irregular tendency to a slowing down of the adsorption from the site of injection in the HT animals accords well with the small and irregular tendency to a reduced level of blood H, but their role in the resistance is insignificant.) As regards the nature of the change enabling the lungs to oxygenate better, caused by HT we know that it can only be a smaller contraction of the bronchioles and of the arterioles (and capillaries?), of the pulmonary circulation. On the question why these HT smooth muscles contract less vigorously to the same H dose than the normal ones do, the following circumstances give some indication: The diminished responsiveness cannot be due to acidaemia alone, though it is true that Eichler *et al.* have shown that acidaemia relaxes the bronchoconstriction, and we found an increased concentration of lactic acid in the HT animals. On the other hand it is also true that the CO_2 tension during the shock is highest in the normal animal which dies, and lowest in the HT animal that survives.

Besides incompensated acidaemia some other changes in the organism or in the smooth muscles in the lungs themselves, must take place, in the HT animals only.

From the facts known so far the following hypotheses are feasible: Adrenaline is known to relax the histamine contraction of the bronchioles (Cameron and Tainer 1936, Daly, Foggie and Hebb, 1940). Evidence of suprarenal hypertrophy (at any rate of the increase of the suprareral/b. w. ratio) in the HT animals is produced (MacKay and Clark 1938). M. Beznák, Korényi and Hajdu (1942) provided experimental proof that acidaemia causes a hypertrophy of medulla and cortex. Korényi and

HAJDU (1942) showed, however, that hypertrophy of the adrenals is not always accompanied by hyperfunction. It is therefore possible that in the diminished response of the smooth muscle in the lung, hyperadrealinaemia concommittant to the acidaemia is the additional factor which only exists in the HT animal, and not in the normal.

It is known that a number of imidazole derivatives, especially arginine are antagonistic to H (ACKERMANN, 1939). HT causes profound changes in the protein metabolism, thus it is possible that amongst the intermediary metabolites such antagonists are liberated.

A very well established fact is that suprarenalectomy increases, and suprarenal cortical hormone decreases, the toxicity of H, and, as we mentioned, there is a suprarenal cortical hypertrophy in the HT animal. But what we know of the mode of action of the suprarenal cortex in counteracting the effect of H, does not support the assumption that it decreases the responsiveness of the smooth muscles to the lung. As opposed to this, we know that it is necessary to the normal contractibility of the arterioles and capillaries.

Yet another hypothetical possibility is that HT damages the smooth muscle of the bronchioles and arterioles, in the lungs, in consequence of which damage they simply cannot contract to H as vigorously as the normal ones do. This assumption is supported by the general failing appearence of the HT animals, by the infarcts and edema seen at autopsy chiefly in the non-resistant HT animals, and by the histological findings. (HEINLEIN, 1937, MERKEL, 1942, JANCSÓ, 1941, TÖRŐ, 1942). These latter indicate severe damage in the blood vessels of the lungs and the heart, as well as in the heart muscle. Although so far this hypothesis seems to be the most plausible, it must be pointed out that these damages, such as infarcts, edema etc., were less pronounced in the resistant than in the non-resistant HT animals. It is of course possible that the damage caused by HT was in the resistant animal just enough to decrease the responsiveness of the resistant HT smooth muscle and in the non-resistant ones it went further, thus reducing the functioning surface of the lungs with the severe haemorrhages.

All these are, however, mere speculations. Only further experiments can decide which of them or yet another, hitherto unforseen, mechanism is the true cause of the decreased responsiveness of the bronchioles and arterioles in the resistant HT animals.

In conclusion, we must warn against any generalisation. The mechanism of H death in other species is different from that in guinea pigs. In those animals therefore, where the H killed them through other me-

chanism than broncho-constriction the HT may not increase the resistance (Remember that CSÁKY and KOVÁCH proved that the HT does not alter in the cat the blood pressure response to H). Moreover, it may be even in the guinea pig that death through bronchoconstriction-asphyxia is only the most general mechanism, but that in some other cases some other vital function breaks down. This may, e. g., be the failure of the right heart. It is possible that in this mechanism HT will have no life-saving effect. Perhaps such animals form the group of guinea pigs whose resistance could not be increased by HT.

SUMMARY.

1.) Comparative determination of the O_2 contents of guinea pigs' blood proved that the cause of the resistance to a s. c. injected LD of H in the resistant HT animals is better oxygenation.

2.) This better oxygenation is due to a decreased contraction response of the broncho- and vaso-constrictor muscles to the same level of H in the blood, since determination of the blood in normal, non-resistant HT guinea pigs showed no significant difference.

3.) The small and irregular relative diminution of the blood H after s. c. H injections, in the HT animals accords well with the small and irregular slowing down of adsorption of H from the site of injection (peritoneal cavity) and with the circumstance that i. c. injected H disappears from the blood equally fast in both normal and HT animals.

4.) The blood lactic acid content of the HT animals is higher than that of the normal ones. During H shock the CO_2 content of the blood is highest in the normal, and lowest in the resistant HT animals. The diminished contraction of the smooth muscles in the lungs cannot therefore be conditioned solely by the acidaemia.

5.) The possible physiological mechanism of the decreased responsiveness caused by HT is discussed. It is pointed out that at present the likeliest explanation is that HT damages the broncho- and the vaso-constrictor muscles which consequently are unable to contract and allow a better oxygenation, and increased blood flow through the lungs, This hypothesis is supported by the autopsy results.

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REFERENCES.

ACKERMANN, D. (1939). *Naturwiss.* **515.**

BARSOUM and GUDDUM, J. H. (1935). *J. of Physiol.* **85.** 1.

BEZNÁK A., KOVÁCH, A. Gy. B., GÁSPÁR-RÁDY, Zs. (1947) *Arch. Biol. Hung.* **17.**

BEZNÁK, M., KORÉNY Z. and HAJDU, I. (1492). *Arch. Intern. Pharm. Therap.* **67.** 242.

CAMERON, W. M., and TAINER, M. L. (1936). *J. of Pharmacol.* **57.** 152.

CODE, C. F. (1937). *J. of Physiol.* **89.** 257.

CSÁKY, T., KOVÁCH, A. (1946). *Arch. Biol. Hung.* **17.**

DALE, H. H. and LAIDLAW, P. P. (1910—11). *J. of Physiol.* **41.** 318.

DALY, DE BURGH I., FOGGIE, P. and HEBB, C. C. (1940). *Quart. J. of Physiol.* **30.** 21.

FICHLER, O., and MÜGGE, H. (1931). *Arch. exp. Path. u. Pharmak.* **159.** 633.

EICHLER, O., and KILLIAN, H. (1931). *Arch. exp. Path. u. Pharmak.* **159.** 606.

EICHLER, O., SPEDA, G. (1940). *Naunyn-Schiedebergs Arch.* **195.** 102.

FIELD, M. E., and DRINKER, C. K. (1930). *Amer. J. Physiol.* **93.** 138.

GREEN, H. H. (1945). *Brit. Med. Bull.* **3.** 692.

HEINLEIN, H. (1937). *Zbl. Path.* **66.** 93.

JANCSÓ, M. (1941). **11.** *Tagung. Ung. Physiol. Ges. Physiol. Ber.* **126.** 475.

KARÁDY, St. (1935). *Arch. exp. Path. u. Pharm.* **180.** 282.

KARÁDY, St. (1938). *Amer. J. Physiol.* **123.** 194.

KARÁDY, St., SELYE H. and BROWNE, J. S. L. (1938). *J. of Immunol.* **35.** 335.

KOKAS F., SARKADY, L., WENT, I. (1938). *Magy. Orv. Arch.* **39.** 408.

KORÉNYI, Z., HAJDU, I. (1942). *Arbeitsphysiol.* **12.** 31.

LAURENSEN, F., and WAHLLÄNDER, H. W. (1938). *Biochem. Ztschr.* **298.** 273.

MACKAY, E. M., and CLARK, W. G. (1938). *Proc. Soc. Exp. Biol. and Med.* **39.** 56.

MERKEL, H. (1942). *Ztschr. exper. Med.* **111.** 98.

NOBLE, R. L., and COLLIP, J. B. (1941). *Amer. J. Physiol.* **133.** 633.

ROSE, B., KARÁDY, St., BROWNE, I. S. L. (1940). *Amer. J. Physiol.* **129.** 218.

RUSZNYÁK, St., KARÁDY, St., SZABÓ, D. (1936). *Arch. f. klin. Chirurg.* **187.** 279.

SELYE, H. (1937). *Science* **85.** 247.

VAN SLYKE, D. (1927). *J. Biol. Chem.* **74.** 659.

TÖRŐ, I. (1942). *Ztschr. mikr.- and Forsch.* **52.** 552.

WILSON, H. (1941). *J. of Physiol.* **94.** 241.

THE EFFECT OF PROLONGED ADMINISTRATION OF l-ASCORBIC AND d-GLUCONIC ACID ON THE BODY-WEIGHT, FOOD, WATER AND SALT-SOLUTION CONSUMPTION OF ALBINO RATS.*

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(With 3 Figures and 2 Tables in the text)

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It is more and more generally accepted (BEZNÁK, BEZNÁK, GÁSPÁR-RÁDY 1941; BACKMAN, 1931) that the physiological and biochemical mechanism of growth is not the same in its whole course, but varies according to age.

Sachs (1874) and Minot (1907) observed that the general property of the absolute growth velocity°° curve is: that it is divided into three periods. The growth velocity increases during the first period, maintains its highest level during the second, and decreases during the third. According to BACKMAN these periods result from the different combinations of three processes: steady growth, acceleration, retardation. In trying to work out an equation for the growth of albino rats, DONALDSON (1924) found that he got the measured and estimated values to tally only if he established two equations for the growth period; one for the 10—80th day and one for the 80—365th day. Consequently, according to DONALDSON, growth takes place in two periods (a faster and a slower).

One of the chief aims of our work was to examine experimentally — by measuring the food and water consumption during the whole growth period — whether the physiological and biochemical mechanism of growth changes during the different stages of life.

The effect of an indispensable nutritional factor has chiefly been

* Part of the results of this work was published at the meeting of the Hungarian Physiological Society, May 22nd, 1941. (BEZNÁK et al. 1941)

°° The absolute growth velocity means the percentual relation between the increase in growth during a given period and the initial-weight at the beginning of the same period.

tested on young animals (by totally eliminating the factor in question). In reality nutrition differs from experimental conditions in these two points: firstly, whatever the mode of nutrition may be, it generally covers the whole life span; secondly, small disequilibria in nutrition take place rather than a total lack of a definite nutritional factor. Recently great quantities of vitamins B_1 and C have been taken, though their effect is known only through relatively short experimental periods.

Our experiments answer the question: What is the effect of prolonged and greatly over-dosed administration of a single nutritional factor (vitamin C) if the diet is otherwise balanced?

The symptoms of C-hyper-vitaminosis have been studied only in the case of such organisms as are unable to synthetise vitamin C (man, guinea pig) and only the acute effect of relatively short experimental periods has been observed. RIETSCHEL (1939) and CENTAMINI (1938) administered vitamin C to rats for longer periods but did not observe its effects.

Apart from observing the effects of the prolonged over-dose of vitamin C, we are also able to account to some extent for their physiological mechanisms and can partly determine which part of the vitamin C molecule is responsible for the effect brought about. D-gluconic acid has many qualities in common with vitamin C (especially that it is also a fairly strong water-soluble hexuronic acid) but it has no anti-scorbutic effect. The effects brought about by vitamin C only may be attributed to the anti-scorbutic redox-group of the molecule, whereas the common effects are caused by the identical qualities of the two compounds, especially their acidity.

METHODS.

Our work was done on male albino rats. The composition of their diet was the following: 25—25% wheat, rye and corn, 15% casein, 3.5% butter, 4% McCollum salt mixture, 1.3% cod liver oil. Our experimental animals were the offspring of mothers living on a complete diet. During the gestation and suckling period the mothers got the above experimental diet, which was also fed to the experimental animals straight after the weaning.

All rats lived in individual cages which made refecation impossible. The food consumption was measured daily with an accuracy of $\pm$ 0.1 g. The rats were supplied with measured quantities of tap water and of a 1% solution of NaCl, given to them in suitable vessels. The remaining fluids were measured each day with an accuracy of 1 ml. From time to time we changed the contents of the vessels and their places in the cage also (the salt where the water had been and vice versa). We tried thus to exclude the possibility of the rats being

influenced by the shape of the vessels or their places, rather than seeking the composition of the fluid itself.

The rats were divided into three groups. The first one received each day through a stomach tube 20 mg ascorbic acid in 0.2 ml distilled water, the second 20 mg d-gluconic acid* in 0.2 ml distilled water, the third one only 0.2 ml distilled water. Each group consisted of ten rats. The experimental period lasted from the 18th of March, 1941, to the 6th of November, 1941, that is, from the 48th day to the 256th day of the rats' life. We did not notice any difference in the behaviour and condition of the three groups. Intercurrent death took place five times, twice in the ascorbic, once in the gluconic acid and twice in the normal control group.

The rats were weighed with an accuracy of $\pm$ 1 g.

Our paper contains the following mathematical formulae: n = the total number of the members of the different groups. M = mean value $= \frac{\sum x}{n}$; $\mu =$ = standard deviation of individual data in a group. $= \frac{\sum \Delta x}{n} 1{\cdot}25$; $\mu_x =$ standard deviation of the arithmetical mean $= \frac{\mu}{\sqrt{n}}$; k = significance of difference between the mean value of two groups $= \frac{M_1 - M_2}{\sqrt{\mu_{x1}^2 + \mu_{x2}^2}}$; correlation coefficient between two values changing simultaneously $= r_{1,2} = \frac{\sum \Delta x_1 . \Delta x_2}{n . \sigma_1 . \sigma_2}$; $b_{2,1} = r_{1,2} = \frac{\sigma_2}{\sigma_1}$ regression coefficient (the magnitude of change of one value corresponding to unit change of the other.)

EXPERIMENTAL RESULTS AND THEIR DISCUSSION.

a) The growth curve.

Figure 1. shows the mean values of our growth curves as well as those of Donaldson (1924.) which we transcribed for the sake of comparison.

As is seen from the Figure, the final mean body weight of the animals receiving ascorbic or gluconic acid is 11.5% higher than that of the normal animals. The mean values of our normal animals are almost identical with those given by the Donaldson curve.

The growth velocity curves (Sachs, 1874) of the three groups are also represented in Figure 1. that is, it shows the percentual correlation between the gain in weight during a 10 days' period and the weight at the beginning of the same period. As Figure 1 shows, the growth velocity curves of the three groups are almost identical.

*Part of the gluconic acid was furnished by Professor Géza Zemplén, for whose kindness we are glad to express our thanks.

Our experiments do not cover the beginning of the growth curves. The extrauterine growth (growth velocity according to SACHS) of the albino rat takes place in three phases: 1.) The quickening of the growth velocity from the 10th day (12 g) to the 30th (28 g). 2.) The attainment of a peak during the 30th to 40th day (28—38 g of body weight). 3.) The slowing down of the growth velocity from the 35—40th day to the 160th day (240 g). To these could be added as a fourth period the phase of steady body weight until the decline of old age.

To decide the question whether ascorbic or gluconic acid had any influence on body weight, we calculated in 10 days' intervals the mean

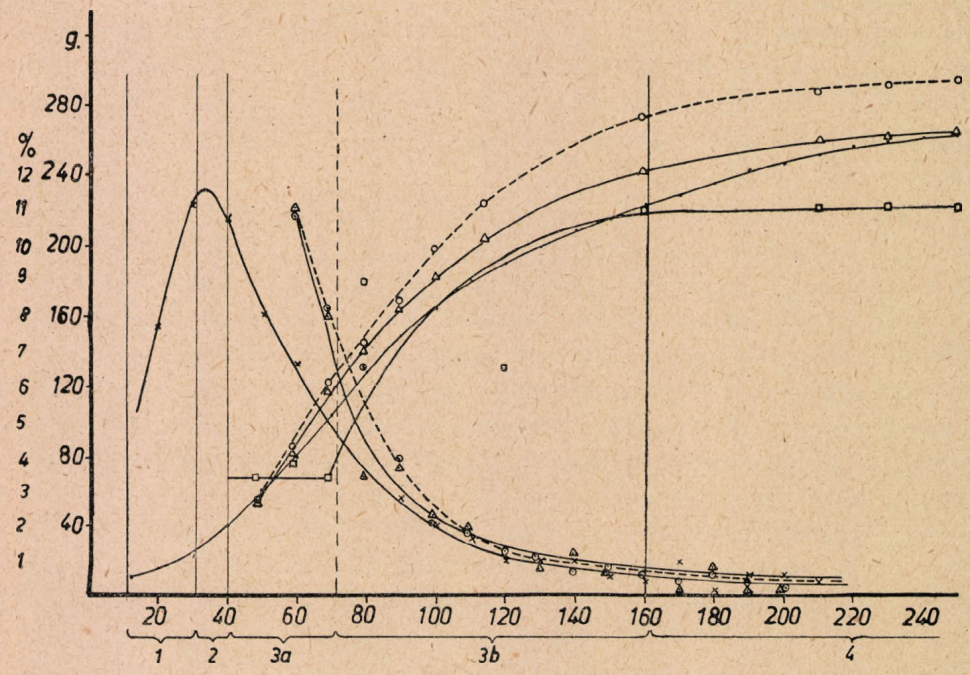

Figure 1. a) The curves of body weight: time of normal △———△, of ascorbic, respectively gluconic-acid fed ○– – –○, and of normal (DONALDSON) •———• rats. x-axis = days of rats' life; y-axis = body weight in gs
b) The absolute velocity of growth of normal △———△, of ascorbic, respectively gluconic-acid fed ⊙——⊙, of normal (DONALDSON) ×——× rats. x-axis = days of rats' life; y-axis = velocity of growth.
c) The difference between the averages of body weights of normal and of acid-fed rats as percentages of the normal b. w. □———□ X-axis days of rats' life; y-axis = 1% difference = 5 mm.
d) The long vertical lines indicate the limits of the phases of growth according to Sachs, respectively according to our experiments.

body weights of the three groups and their significant differences. Figure 1. shows these differences in per cent. According to these calculations the mean body weights of the groups receiving acid was only 3.5% higher than that of the normal groups till the 70th day (k = 0.7—1.1. After this the difference becomes suddenly greater (9—11.5%) and remains on this level till the end of the experiment.

The third period of the growth curve as devised by SACHS should — according to our experiments — be divided in two: in the first the decrease of the growth velocity takes place quickly, in the second more slowly. The first period extends in our case to the 70th day, in that of DONALDSON to the 80th day. We might call the first period the 3a phase and the second the 3b phase. As was mentioned introductorily DONALDSON expresses growth of rats with two equations, the first covering growth in the first 80 days, and the second, growth from the 80th day onwards. Accordingly, DONALDSON's first period includes SACH's first, second and our 3a phase, his second period our 3b and 4th phase.

The fact that there was no difference in the mean values of body weights in normal and acid-receiving groups till the 70th day, when there appeared suddenly a difference of 9%, which increased gradually to 11.5%, admits of two explanations. It is possible that at this juncture some change in the metabolism of growing rats takes place, in consequence of which some rats attain a greater body weight. The other possiblity is that the difference is caused by the acid administration. In the first instance the greater mean body weight of the groups receiving acid must be attributed to haphazard group formation. The significant difference of the mean body weight values is only 2, so that we cannot exclude with an absolute certainty the possibility of haphazard group formation. Still, if we take into consideration a) that the two acid-receiving groups grew suddenly and simultaneously, from the same time onwards, better than the normal group and b) that, in spite of the fact that the body weight of the gluconic acid-receiving groups was the smallest in the beginning, yet they grew better than normal and better even than the ascorbic acid animals, we must consider it more likely that the difference in weight was caused by the acid administration and not by haphazard group formation. Again, the acid-administration might cause better growth just from that point onwards in two ways: a) either the acid must be given during several weeks to develop its influence, or b) the changed metabolism reacts differently to acids. The circumstance that

growth of normal rats slows down considerably from that point, argues in favour of the second hypothesis.

Thus C hyper-vitaminosis has no specific influence on growth, and the small effect observed is the same in the case of another non anti-scorbutic hexuronic acid. Contrary to this, B_1 hyper-vitaminosis on a SHERMAN CHASE 515 complete diet has a definite, strong influence on growth, so that the B_1 hyper-vitaminotic animals grew to 450 g, 25% more than the final weight of our biggest rats (360g) (WATERMAN and AMMERMAN 1938).

b) The food, water, and 1% salt-solution consumption.

Growth consists of retaining in the body some elements of the food and water consumed. The next question to be considered therefore is the following: What is the connection during the different phases of growth between the velocity of growth and the quantity of food, salt and water consumed. Though the greater part of the body is composed of salt solution, attention in the past was mainly directed towards food consumption rather than water or salt solution consumption.

As regards the relation between growth and food consumption, RUBNER's law of continuous energy-loss (1908) and the LUSK-WILSON rule (1902) were generally accepted. According to the first, the energy quantity (for which the quantity of food consumed may be substituted) needed for the new-born to double its weight is the same whether it grows slowly or quickly. In other words, the plus body weight during growth is always the same percentage of the food consumed — independent of growth's being a slow or a quick process. The LUSK-WILSON rule is substantially the same as RUBNER's law — though it is more accurate: „Animals of the same breed and age retain a definite and constant quantity of food consumed during the normal development of the young" (l. c.). According to these two rules, bigger and faster growing animals do not accomplish this (faster and greater growth) by greater retention but by eating more.

Our experiments are capable of controlling these two rules with the aid of probablity calculation. Figure 2 shows the food consumption. The mean consumption counted per 10 days and per animal increases rapidly during the first 90 days, then after a slight elevation remains on a constant level. Accordingly, the food consumption shows a slight elevation parallel with the relatively quicker growth during the 3a period. During the 3b period food consumption hardly changes, though growth still continues. During the 4th period of the growth

curve, when body weight is constant, food consumption diminishes. It is seen from this Figure that the food consumption of the animals receiving either ascorbic or gluconic acid is practically the same, during the whole growth curve, as that of the normal group. There

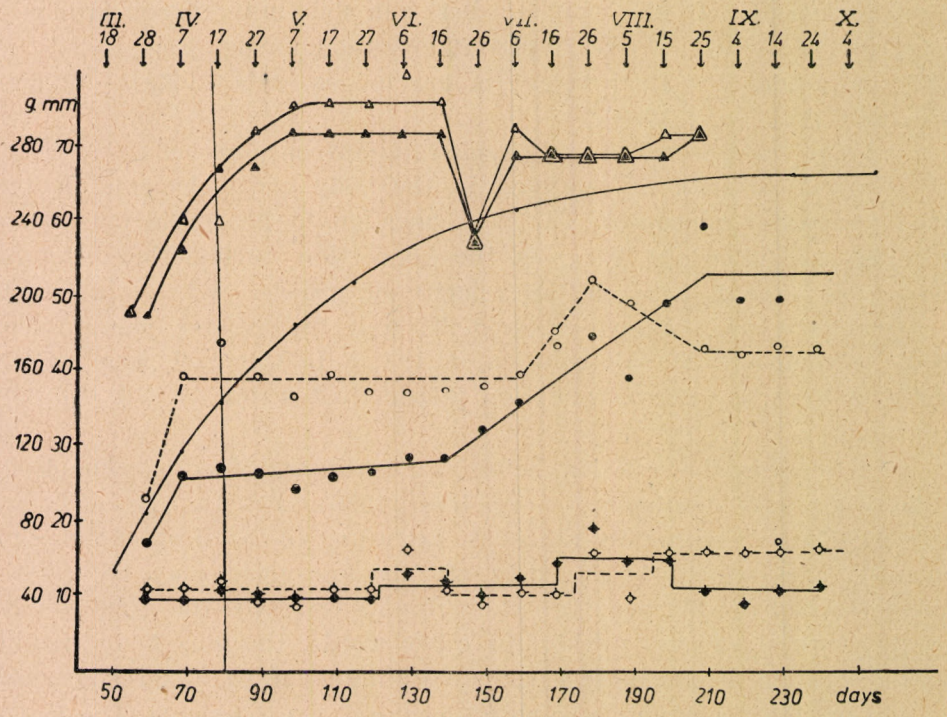

Figure 2.

10 days rat.
- Food consumption — normal ▲——▲ / acid-fed △——△
- 1% salt „ — normal •——• / acid-fed ○ - - - ○
- Water „ — normal ●——● / acid-fed ☼——☼

Vertical line: end of the 3a period of growth.
Growth curve •——•

is nowhere a significant difference in the food consumption of the animals belonging to different groups.

That RUBNER's rule is not valid for all parts of the growth curve can be shown by simple calculation. During the 3a period of growth,

that is from the 48th to the 68th day, the animals grow from 55 to 115 gs; in other words, they somewhat more than double their weight. During that time they consume 260 gs. of food. Therefore, to produce 1 kg. of growth, 4270 gs. of food are needed; that is, the 61 gs. of plus body weight represents 23% of the food consumed. In RUBNER's own experiment this was 36%. However, during the 3b and the following 4th period of growth, that is, from the 68th to the 208th day, the animals grow from 140 gs. to 269 gs. (that is, 129 gs, almost doubling their weight) and consume during that time 2260 gs. of food. For 1 kg. of plus body weight the food consumption is 17,520 gs, so that growth represents only 5.8% of the food consumed. The RUBNER rule can therefore not be valid for the whole length of the growth period, but (if it is valid at all) only for a small part of it, that is, the 3a sub-division.

As growth continues, food consumption remaining the same, it is evident that food consumption per unit weight and unit time, must diminish. The part of food consumption utilised by growth during the different periods is shown in Figure 3. It is apparent from this Figure that growth has to be divided here too, into two parts .During the first (till the end of the 3a period) the part utilised by growth, counted per kg and day from the food consumed, per kg and day, diminishes quickly, during the second (in the 3b and 4th period) slowly.

Whether the LUSK-WILSON rule is valid or not, can also be determined by our experiments. If the food consumed is built up in the body at the same rate by quickly and by slowly growing animals, then there should be no correlation coefficient between the growth in gs during identical periods and the precentual relation between growth and food consumption (the latter is called efficiency of growth). In our 30 animals the growth from the 48th to the 60th day was 65 gs ($\mu = \pm$ 11.7) and growth as percentage of the food consumption was 25% ($\mu = \pm$ 3.3). In WILSON's experiment this was 18—19%. The correlation coefficient (r) between the two groups of data is 0.9, $\pm$ 0.034, almost the maximum. (The same is obtained by using for calculation only the third group). Therefore WILSONS' rule is not valid even during the 3a period of growth. From this again it follows that the RUBNER rule cannot be valid either. The faster growing animals grow faster also because they build up in their bodies a greater part of the consumed food than do the more slowly growing ones. If a rat grows more quickly by one g each 20 days that means a corresponding greater retention of 25% of the food consumed. The contradiction between RUBNER, WILSON and our

results is probably caused by the circumstance that they experimented on small numbers of animals and only for a short time. (For example, WILSON observed 5 pigs from the same litter for 14—16 days.)

As is seen from Figure 2, the increase in food consumption stops sooner than the increase in body weight. During the period in which food consumption remains unchanged growth can evidently only be

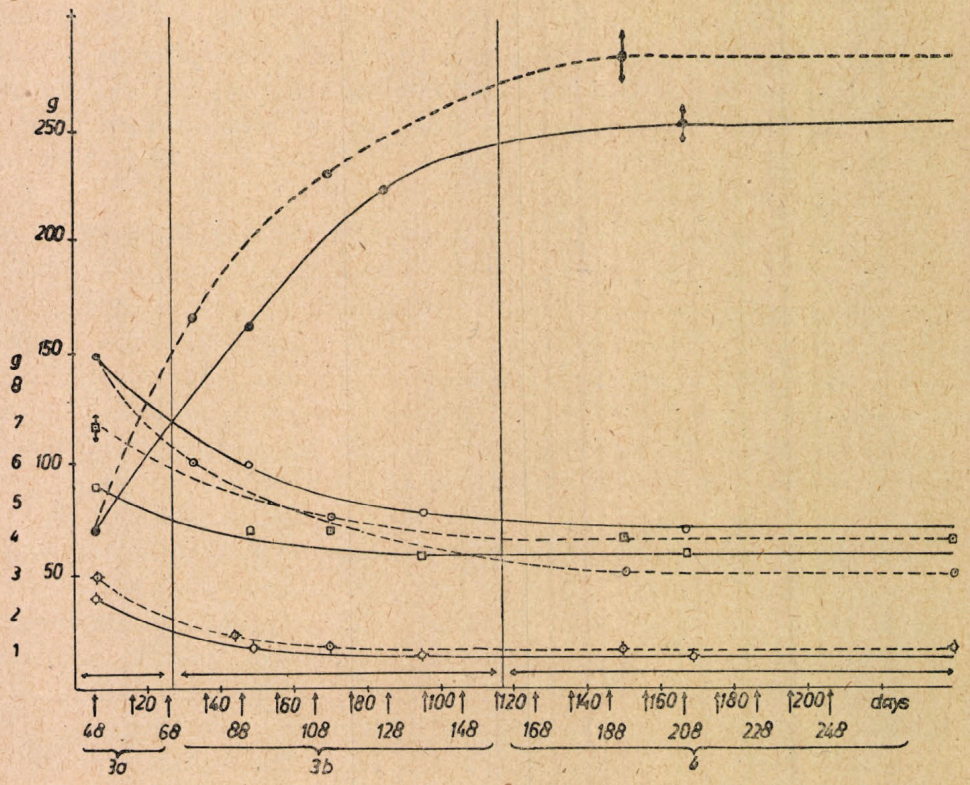

Figure 3. Increase of body weight/kg-day normal •——•, acid-fed •- - -• rats. Food consumption/kg-day normal ○——○, acid-fed ⊙——⊙ rats. 1% salt solution consumption/kg-day normal □——□, acid-fed ⊡——⊡ rats. Water consumption/kg-day normal ⌾——⌾, acid-fed ⊙——⊙ rats. Short vertical arrows show the standard deviation of the average. x-axis upper line = days of experiment; lower line = days of rats life.

caused by a greater growth efficiency. In attaining a higher final body weight a third circumstance may also play a role: that is, the point on the growth curve from which the increase in food consumption

stops. If this point is reached earlier by faster growing animals (possessing greater efficiency of growth), then the quantity of food the animal is capable of building up in his body plays a more important role even during the 3a period than the increase in food consumption, in the attainment of the final body weight. This can also be determined by employing correlation calculation. The value of r between the efficiency of growth and the length of the period of increased food consumption is 0.51 ± 0.05. That means that,admitting great individual variability, the period of increase in food consumption is shorter in the faster growing animals possessing greater efficiency of growth. This shows the great significance of growth efficiency. The sooner the growing animal attains the period of constant food consumption, the sooner begins, and the longer will be the period during which it grows only because it retains and builds up in its body a greater percentage of the food consumed.

Though from our experiments it seems indubitably proved that the retention of a greater percentage of the food consumed plays an important role in the efficiency of growth, it is nonetheless possible that greater food consumption also may be important. HOPKINS (1912) published results showing that those groups of rats that ate more per unit weight grew better. Nevertheless these better-growing rats used up, according to our experiments, a greater percentage of the food consumed for growth. To decide this question we calculated the correlation coefficient between velocity of growth and the amount of food consumed. The correlation coefficient (r) was found to be 0.55, $= \pm 0.13$; that is, there is a certain tendency in the faster growing animals to eat more, but the correlation between velocity of growth and amount of food consumed is not so close as between velocity of growth and efficiency of growth. This latter is, therefore, the more important factor in determining the velocity of growth. Our conclusion is in agreement with that of PALMER and KENNEDY 1930, who with different experiments came to the conclusion, that „Individual variation in efficiency quotient, is a major, if not the controlling cause of individual variation in gain in weight of animals on the same diet."

Animals receiving acid attain an 11.5% higher body weight than the normal ones. How does this difference in body weight originate? One possibility is that hexuronic acids increase the efficiency of growth; the second, that, not influencing efficiency of growth, they increase the food consumption; the third, that, having no influence on either, they increase water retention. Table 1. shows the results calculated

from all the four periods of growth. According to this, the animals that receive acid (and grow 11.5% more) consume 5% less than normal ones to build up the same body weight. Therefore the increase of growth in dry weight is occasioned in some rats by the fact that acid increases efficiency of growth. As the value of k is low — 1.6 — in other animals this mechanism does not work. In these the food consumption has to increase, leaving efficiency of growth unchanged. The fact that there is a tendency — also with a low value of k — in the acid-

TABLE I.

Food consumed in g. -s. necessary to build up 1000 g. -s of living weight, calculated from all the data obtained during the 3a, 3b and 4-th phases of growth

Treatment	No.	Mean value μ	μ_x
Ascorbic acid	9	$M_{a.} = 7599$	
Gluconic acid	10	$M_{g.} = 8818$	
Ascorbic + gluconic acid	19	$M_{a.g.} = 8709 \pm 185$	42
Normal	10	$M_{n.} = 8016 \pm 711$	225

Differences	g	%	k
$M_{a.g.} - M_{n.}$	—369	—4.5	1.6

receiving (bigger) animals to eat more (see Figure 2.) is in accordance with this hypothesis.

Our results regarding the consumption of 1% salt-solution and of water, are also shown in Figure 2. From these results the following conclusions can be drawn: the rats on this diet seek salt-solution and drink only small amounts of water. During the experimental period there were some days when they drank no water at all, but they always consumed, though in changing quantity, the salt-solution. The other important difference we observed in their consumption of salt-solution and of water is that water consumption is almost constant, independent of the growth of the rat. Whereas the rat quadruples its weight from the 48th to the 120th day, its water consumption is almost constant, independent of the growth of the rat. Contrary to this, salt-solution consumption runs somewhat parallel to the growth curve. From the 48th to the 60—70th day, during the 3a period, salt-solution consumption

increases with growth; from there on it remains constant to the 150th day, although body weight is doubled during that time. After this salt-solution consumption attains a higher level, although growth has stopped.

The effect of acid administration on salt-solution and water consumption is to be seen in Figure 2. Acid administration has no effect on water consumption during the 3a and 3b period, but after the completion of growth, during the 4th period (constant body weight of 240—250 gs) the water consumption of acid-receiving animals increases by 50%. The acid-receiving animals consume salt solution at a greater rate during the 3a period than the normal animals do. During the 3b period salt-solution consumption is constant although growth continues. During this period there is no difference in salt-solution consumption between acid-receiving and normal groups. The increase in salt solution consumption after the 3b period is greater in normal than in acid-receiving animals. Salt consumption apparently remains on this higher level during the whole fourth period.

As the mass of the body is water for the most part, growth can be interpreted, as the building up of such molecules as are capable of more and more water retention. The answer to the question whether the 11.5% higher body weight is caused by greater fluid retention or greater fluid consumption, is given in Table II. From this it is apparent that to build up 1 kg living weight, acid-receiving animals take up 15% more fluid than normal ones; that is, influenced by acids, the animals retain less fluid. As water consumption is almost the same in all groups, the difference refers primarily to salt consumption.

Table II. shows these results during all four periods of growth. To form a picture of the influence of acids during each period we calculated on 6 day averages the salt-solution consumption per day and per kg body weight. Acid-receiving animals have consumed at the end of the 3a period 33% more ($k = 2$), at the beginning of the 3b period 42% more ($k = 2.3$), at the end of the 3b period 12% more ($k = 1.1$) and at the beginning of the 4th period 3% more ($k = 0.2$) salt-solution than normal ones. Taking into account the results of the food consumption, we come to the conclusion that the higher final body weight is caused during the 3a period by greater salt-solution consumption and unchanged retention, and during the later periods by greater salt-solution retention.

There are some observations which indicate that there is a greater need of salt in organisms with suprarenal insufficiency. Richter (1938, 1939). We calculated, therefore, the correlation coefficient between the

weight of the suprarenals and the average daily salt consumption and found $r = \pm 0.185 \pm 0.13$ This means that among the animals with large suprarenals there are some which drink less salt than others with small suprarenals, but that this tendency is influenced by many different circumstances.

It is well known that the decreased functioning of the suprarenal cortex is accompanied by cessation of growth, and in adult rats by the diminishing of the restitutional metabolism. We calculated, therefore, the correlation coefficient between the suprarenal weight and the efficiency of growth in individual rats and found $r = 0.7 \pm 0.01$. This value is rather high and it means that 0.28% increase in the efficiency

TABLE II.

Fluid intake (1% salt solution + water) in g-s, necessary to build up 1000 g-s of living weight, calculated from all the data obtained during the 3a, 3b and 4-th phases of growth

Treatment	No.	Mean value μ	μ_x
Ascorbic acid	9	$M_{a.} = 8600$	
Gluconic acid	19	$M_{g.} = 8818$	
Ascorbic + gluconic acid	19	$M_{a.\,g.} = 8709 \pm 1852$	426
Normal	10	$M_{n.} = 7521 \pm 1478$	468

Differences	g. g	%	k
$M_{a.\,g.} - M_{n.}$	+1188	%15	1.9

of growth corresponds to 1 mg of suprarenal plus, or 1.7 mg greater suprarenal corresponds to 1% increase in the efficiency of growth. The biochemical mechanism of this definite correlation may be the following: 1.). Either the greater suprarenal corresponds to greater hormone secretion and that to greater efficiency in growth, or 2.) as we know from DONALDSON'S observations that suprarenal weight varies according to the body weight, this rule may also hold good for the suprarenal as part of the body (that is it attains greater growth by greater efficiency). The first mechanism is the more probable, as the correlation coefficient between the body weight and efficiency is 0.9, whereas between the efficiency of growth and suprarenal weight only

0.7. The fact that growth is caused by greater retention is more evident in the case of the body and its tissues (connective tissue, muscle) than in the suprarenal. Moreover, if the suprarenal is affected by illness growth stops; that is, the efficiency of restitution diminishes.

SUMMARY.

1. A comparison of the alteration in the growth curve caused by administration of hexuronic acids with the curve showing food, salt-solution and water consumption supports the hypothesis that the physiological and cell biochemical mechanism of growth in mammals changes in the different phases of growth . In the growth curves of the mammals one can differentiate at least five such phases.

2. Neither the RUBNER nor the LUSK-WILSON rule is valid for the velocity of growth. The velocity of growth depends chiefly on the direct correlation between velocity and efficiency of growth and the indirect relation between the length of the period of increased food consumption and the efficiency of growth.

3. Among the animals with large suprarenals there are more that drink less salt solution than among those with small suprarenals. There is a strong positive correlation between the size of the suprarenal and the efficiency of growth. The variations in consumption of salt-solution and of water bear no relation to one another.

4. The body weight of those rats which receive from their 48th to their 250th day, a daily dose of roughly 200 mg/kg l-ascorbic or d-gluconic acid, is 11.5% greater than that of normal rats. Statistical calculations and the careful weighing of the experimental elements make it likely that the difference is caused by the administration of the acids. Besides stimulating growth, ascorbic and gluconic acids act in identical ways on the consumption of food, salt-solution and water. These effects, therefore, are not due to the anti-scorbutic radical of the ascorbic acid but probably to the acid group present in both acids.

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LITERATURE.

BACKMAN G. (1931). Erg. Physiol. **33.** 904.

BEZNÁK A. B L. BEZNÁK M., and GÁSPÁR Zs. (1941). Physiol. Ber. **126.** 469.

CENTAMINI. CÍT. (1938). Fermente, Hormone, Vitamine. Ammon Dierschel Leipzig p. 321.

Donaldson H., Dunn E. H., Watson Ch. (1924). Boas Anniversary Volume. NewYork 1906. Cit: Donaldson The Rat. Philadelphia. 1924.
Donaldson H. (1924). The Rat. Philadelphia.
Hopkins F. G. (1912). J. Physiol. **44.** 425.
Minot (1907). The Problem of Age, Growth and Death. III. Popular Science Monthly. **71.** 193. Cit: Mendel. Erg. Physiol. **15.** 124. 1916.
Palmer Z. S., Kennedy, e. (1931). J. Biol. Chem. **90.** 545.
Richter C. P., Eckert J. F. (1938). Endocrinology. **22.** 214.
Richter C. P. (1939). Endocrinology. **24.** 367.
Rietschel H. (1939). Klin. Wschr. *II.* 923.
Rubner M. (1908). Das Problem der lebensdauer etc. Berlin. Cit.: Lusk G.: The. Science of Nutrition. Philadelphia. 1928. p. 567.
Sasch J. (1874). Arb. Bot. Inst. Würzburg. **1. 99.** Leipzig. Cit. Backman l. c.
Waterman and Ammerman. (1938). Cit. R. R. Williams. Erg. Vit. Forsch. **1.** 230.
Wilson M. B., Sanford, L. C. (1902). Am. J. Physiol. **8.** 197. Cit.: Lusk G.: The Science of Nutrition. p. 565—568.

THE EFFECT OF PROLONGED ADMINISTRATION OF l-ASCORBIC AND d-GLUCONIC ACID ON THE WEIGHT, FAT, PROTEIN, GLYCOGEN AND VITAMIN-C CONTENT OF SOME ORGANS OF ALBINO RATS.*

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With 1 Figure and 3 Tables in the text.

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In our previous paper we described (BEZNÁK, BEZNÁK and GASPÁR-RÁDY 1947) the growth, food, salt and water consumption of albino rats and the effects brought about by l-ascorbic or d-gluconic acid administration. It was probably due to these acids that the rats receiving them attained 11.5% higher body weights than normal rats. No difference in the action of ascorbic and non anti-scorbutic gluconic acid could be observed. Therefore the effect could not be attributed to the anti-scorbutic redox group but probably to the acid group present in both acids.

In this paper we publish the observations we made to decide what part the individual organs play in the development of greater body weight and whether ascorbic acid has any specific influence on the composition of the tissues.

As to methods, we have to add to those already published in our first paper the following particulars:

The animals were bled to death and their organs measured in closed vessels with an accuracy of $\pm$ 1 mg a few minutes after their death. The fat of the connective tissue was considered as a separate organ. After skinning the animals

* Part of the results of this work was published at the meeting of the Hungarian Physiological Society October 3d, 1942. (BEZNÁK et al. 1943). The work was aided by a grant from the Széchenyi Scientific Society.

we removed the subcutaneous fat as completely as possible, and the fat found between the voluntary muscles was also carefully removed. The fat deposits in the cavities were gathered and measured in the following groups: genital, perirenal, mesenterial and inter-muscular fat. The glycogen content of the organs was de-

TABLE I.

Weight of some organs of normal rats and rats receiving ascorbic or gluconic acid.

	Normal			%	k	Gluconic acid			%	k	Ascorbic acid		
	No	M	μ_x			No.	M	μ_x			No.	M	μ_x
Spleen mg.	8	522	± 38			8	582	± 26			9	1045	± 91
				+11	1·3				+ 80	4.4			
									+100	4.9			
Thymus mg.	8	365	± 26			8	503	± 29			9	443	± 46
				+38	3.5								
									+ 22	2.9			
Connective tissue fat g.	8	17					22					21	
				+29	2.6								
									+24	1.9			
Heart mg.	8	932				8	955				9	915	
Liver g.		8.26					9.37					9.00	
				+13	1.8								
									+9	1.2			
Kidneys g.	8	2.16				8	2.35				9	2.31	
Testes g.	8	2.37				8	2.53				9	2.54	
Suprarenals mg.	8	46.4				8	45.4				9	47.4	
Thyroids mg.	8	23.1				8	26 5				9	25.5	

termined by the method of GOOD, KRAMER and SOMOGYI (1935), the fat content according to SOXHLET, and the protein content as N by the method of PARNAS-WAGNER. The ascorbic acid content was determined in a trichloroacetic extract of the organs 40 seconds after their removal with dibromophenol-indophenol.

a.) Weight of the Organs.

Table I. shows the mean weights of the organs measured in g or in mg. In the cases where there was a difference in the mean values we

calculated the standard deviation of the mean values (μ) the differences of the mean as percentage of the normal mean value (d %) and the significance of difference of the means (k). As is seen from Table I, the two acids caused the following changes in the weights of the organs: As a result of the ascorbic acid administration the spleen of the animals is 100% greater than the normal mean value, (k = 4.85).

This effect is therefore a very frequent and constant result of ascorbic acid administration. The spleens of animals receiving gluconic acid show no change compared with normal ones. The thymus is greater both in the animals receiving ascorbic acid and those receiving gluconic acid, in the first instance with 38%, k = 2.9 and in the second with 35%, k = 3.5. The weight of the thymus is therefore

TABLE II.

Composition of the liver and the muscle.

	Normal	% k.	Gluconic acid	% k.	Ascorbic acid
Liver glycogen	2.84 ± 0.32	−34 3.0	1.87 ± 0.85	1.3	1.40 ± 0.54
				−51 6.6	
Muscle glycogen	0.32 ± 0.06		0.27 ± 0.03		0.31 ± 0.09
Liver protein	20.80 ± 0.53		20.93 ± 0.85		20.76 ± 1.14
Muscle protein	21.30 ± 1.06		21.48 ± 1.37		21.78 ± 0.98
Liver fat	10.61 ± 1.79		10.44 ± 3.38		10.41 ± 1.70
Muscle fat	8.56 ± 2.04	+23 1.2	10.52 ± 4.26		10.55 ± 4.22
				+23 1.2	

in more than two-thirds of the ascorbic- and gluconic-acid-receiving animals ⅓ greater than the normal mean value. There is an increase in the amount of connective fat in both ascorbic acid, 29%, k = 1.9 and gluconic acid, 30%, k = 2.6 receiving groups. The difference is significant in the case of genital fat, whereas the other fatty deposits hardly change at all. There is no difference in the weight of the heart, the liver, the testicles, the suprarenals and the thyroid gland.

b.) Composition of the Organs.

Table II. shows the results of the determination of glycogen,

protein and fat in the liver and muscles of the three groups. We see from this Table that the only definite change is the 50% diminution of liver glycogen in both ascorbic and gluconic acid groups. The change refers to the glycogen content only, for the protein and fat content of the livers remains the same. Neither is there any change in the glycogen and protein content of muscles.

The vitamin-C content of liver and suprarenals is shown in Table III. There is 16% more vitamin-C in the suprarenals and 25% more in the liver of rats receiving ascorbic acid. The k value of the differences in the mean values attains 2. In the case of gluconic acid, the

TABLE III.

The vitamin C content of the liver and the suprarenals in mg%

	Normal	% k	Gluconic acid	% k	Ascorbic acid
Liver	16.40 ± 2.94		16.68 ± 4.27	+16 2·0	19.00 ± 2.02
Suprarenals	221.62 ± 56. 95		204.08 ± 61.67	2·6	278.33 ± 47.71
				+25 2·1	

ascorbic acid content of the liver decreases about 10%, so that the increase in vitamin-C content in ascorbic-acid-receiving animals is proportionately even greater (k = 2.6).

DISCUSSION.

Where one can apply probability calculation, our results fall into two groups. In the first, the calculation of the significance of the difference can be applied directly to the difference of the means. The results are the following: 1.) The weight of the spleen is unquestionably 100% greater in the ascorbic-acid-receiving animals, k = 4.9. 2.) The weight of the thymus increases in both ascorbic acid and gluconic acid groups, k = 2.9, and 3.5. 3.) In confirming the earlier results of Mosonyi (1934), we find that prolonged vitamin-C administration causes an increase of 20% in the ascorbic acid content of the suprarenals, k = 2.1, and about the same increase in the ascorbic acid content of the liver, k = 2.0. 4.) The glycogen content of the liver decreases in both ascorbic and gluconic acid-receiving groups. The decrease is 51%

of the normal mean value in the case of ascorbic acid, k = 6.6 and 34% in the case of gluconic acid, k = 3.0. 5.) The glycogen content of the muscles of gluconic-acid-receiving animals decreases by 16% whereas that of the ascorbic-acid group is unchanged. 6.) In none of the groups does the protein content of liver and muscle show any change. 7.) The fat content of the liver also remains unchanged. 8.) This calculation can also be applied to decide whether the 11.5% higher body weight of the acid-receiving groups also refers to the fat-free body weight, or if it is caused only by the greater accumulation of fat.

BACKMAN (1931) is right in pointing out that in the estimation of growth the mistake is often committed of paying no attention to the fat content. Therefore when one reads about growth it often does not mean growth but obesity. Our experiments prove that the amount of fat in the connective tissues increases specifically as a result of acid administration. To decide whether the fat-free body weight also increases in the acid-receiving groups, we calculated the mean values of the fat-free body weight in all groups. This was also found to be 8% higher in both acid-receiving groups, k = 1.7. In our previous paper we enumerated the circumstances under which it was more probable that the higher final body weight of acid-receiving animals was not due to chance group formations but to the acid administration itself, in spite of k being only about 2 (BEZNÁK *et al.* 1947). All we said there is also valid in the case of fat-free body weight but, as k is even lower here, to a smaller extent. Acid administration, therefore, also increases fat-free body weight with great individual variations.

The extent to which parts of the fat-free body take part in the increase is determined by the calculated correlation coefficients. We got values round 0.7—0.8, confirming earlier and similar experiments by DONALDSON. It seems, therefore, that in producing the surplus body weight each organ correlated to the body weight takes part evenly.

The simple calculation of the significance of difference, k, could not be used to decide the results in the three following cases: 1.) The fat content of the connective tissues increases in both acid-receiving groups by 26%. 2.) Both ascorbic and gluconic acid cause an increase of 23% in muscle fat. 3.) The weight of the liver is 8% higher in ascorbic acid, and 13% higher in gluconic acid-receiving groups.

The fat content of the connective tissues shows the following particulars: The fat of connective tissues was 17 g, $\mu_x = \pm$ 0.7 g. in normal, 21 g, $\mu_x = \pm$ 2.0 g in ascorbic, and 22 g, $\mu_x = \pm$ 1.8 g. in gluconic-acid-receiving animals. The difference between normal and

gluconic groups is 30%, k = 1.9. Therefore there is a certain probability that the approximately 30% greater fat content of ascorbic and gluconic acid-receiving groups is caused by acid administration.

To decide the question with greater certainty, we calculated first in every animal, regardless of group, the correlation- and regression coefficient between body weight (w) and connective fat weight (f) The correlation coefficient was found to be r_{wf} — 0.78 ± 0.77, and the regression coefficient b_{fw} = 0.135. It follows from the high value of these coefficients that there is a definite relation between the increase in body weight and that of the connective fat. As ascorbic or gluconic acid administration increases the growth of the body, it had to be decided whether the greater fat content of these animals was caused by their being bigger, or whether acid administration has a particular

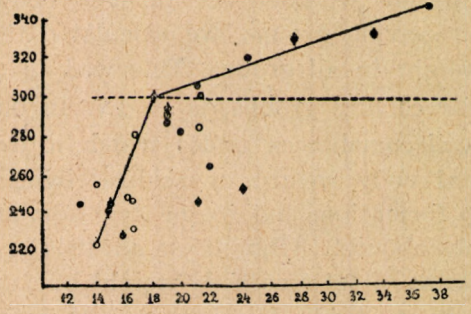

Figure 1. — x-axis connective tissue fat in g-s, y-axis body weight of rats in g-s. ○ normal animals; ● ascorbic acid animals; ◐ gluconic acid animals.

influence on connective fat. We therefore illustrated in a rectangular coordinate the relation between body weight and connective fat, marking differently the rats of different groups. (Figure 1.) The Figure shows that there is no normal rat bigger than 295g and possessing more than 21 g connective fat, (r_{wf} = 0.67 ± 0.16, b_{fw} = 0.061 ± 0.061). There are two important circumstances in the case of ascorbic and gluconic acid-receiving animals: 1.) Regardless of group, both ascorbic and gluconic acid-receiving animals fall into two categories: the weights of the first fall into the range of normal animals; in the second they are bigger. The first important result is, therefore, that both ascorbic and gluconic acid have the same influence on the relation between body weight and connective tissue.

In the case of acid-receiving rats whose body weight falls into the normal range r_{wf} = 0.310 ± 0.031, b_{fw} = 0.050 ± 0.050; in the bigger

rats the same values are $r_{wf} = 0.85 \pm 0.1$, $b_{fw} = 0.33 \pm 0.04$. Since in the rats which are heavier than the biggest normal ones the correlation coefficient between body weight and connective fat is very high (the highest of all: 0.85 ± 0.1), the conclusion is warranted that these rats are bigger because they contain more fat. As these animals can be found only in the ascorbic and gluconic groups (in the same proportion and irregularly) it is certain that this effect is caused by acid administration. The fat content of the connective tissue is therefore in the ascorbic and gluconic groups greater not only because these animals are bigger and fat is a function of body weight, but also because *both acids increase the fat deposition in the connective tissue.* The same conclusion is reached by the following: The correlation coefficient of body weight in those acid-receiving rats which fall in the normal range is low and its variability is high ($r_{wf} = 0.31 \pm 0.31$, $q_{fw} = 0.05 \pm 0.05$). 0.061 ± 0.016 g plus fat corresponds to 1 g body weight gain in normal and 0.05 ± 0.05 g in those acid-receiving animals which fall in the normal range. Similarly the average fat content of normal rats is 17 g, that of acid-receiving rats having the same body weight 18 g. There is hardly any difference in these values, $k = 0.24$.

In all acid-receiving animals, including those bigger than normal, the weight of the fat is 15%, $k = 1.7$ higher than the mean value of normal rats. From this *it is certain that in some animals (40% of all the acid-receiving animals) the obesity was great and that such animals appeared only in the groups treated with acid.*

The k-value of the increase in muscle fat is 1.2; that is, the individual variations are very great. The cause of this is probably that the amount of fat to be seen between the muscles varies and though we freed muscle from fat as completely as possible, different quantities remained. Fat increased probably in these connective cells also. KUSIMOTO and NAZAYUSU (1936) have shown that if they fed fat coloured with sudan, this fat accumulated in these connective cells, but not in the muscle cells themselves.

Do our experiments throw light on the physiological mechanism of obesity? Both ascorbic and gluconic acid cause an increase in fat in the connective tissue. Hence it could not have been the anti-scorbutic radical which effected this change (as this factor is lacking in the gluconic acid). The effect must, therefore, be attributed to identical parts of the two molecules. We think it probable that obesity is caused by the acidaemia induced by the administration of hexuronic acids. The acidaemia is caused partly by the hexuronic acids themselves and partly

by acid intermetabolites, regardless of their molecular structure. We found no experiments in the literature proving that an increase of connective fat results from a diet causing acidaemia. FAZEKAS (1939) observed in rabbits that prolonged administration of ammonia (which also causes acidaemia) produced growth and (statistically uncontrolled) obesity. Our experimental results differ from those of FAZEKAS inasmuch as he got obesity by administering toxic doses of ammonia whereas we produced it under physiological circumstances, by small doses of relatively weak acids which normally occur in the diet also. It seems that our results give the first proof of the fact that *a nutritional habit in which organic acids are in surplus amount causes obesity.*

Our conclusion, that ascorbic acid causes obesity through acideamia, is only in apparent contradiction with the observation (SHEPPARD and MCHENRY 1939) that scorbutic guinea pigs contain more fat than their normal controls. During scorbut there is a pronounced acidaemia (MCCLENDON, MEYSENBURG, ENGSTRAND, KING 1919). The conclusion that the effects of ascorbic or gluconic acid administration are brought about through acidaemia is supported by the decrease in liver-glycogen. We know from the experiments of ELIAS (1912) and ELIAS and KOLBE (1913) that liver glycogen is very sensitive to acid administration and is mobilized by the smallest decrease in the reserve alkali. We believe, therefore, that the decrease in liver glycogen is caused by ascorbic and gluconic acid through their being acids. Thus the contradition that ascorbic acid increases liver glycogen in scorbutic and sometimes in normal animals can be explained. Scorbut itself (SHEPPARD et al. l. c.) causes acideamia and, through it, decrease of liver glycogen. By the administration of ascorbic acid the scorbut is cured, the acidaemia decreases and, in consequence of this, the liver glycogen increases. If we give more ascorbic acid than is necessary to meet the demands in the antiscorbutic principle (as in our case) the effects of ascorbic acid, as an acid, appear. That is why we get a decrease of liver glycogen in consequence of the prolonged administration of small doses of vitamin-C (and d'ALLESSANDRIA and HAAS (1943) in consequence of the administration of *great* doses of vitamin-C for a short period).

We calculated, as above, the correlation coefficient between the liver weight (l) and the connective fat (f). We found a high correlation between these values, $r_{lf} = 0.75 \pm 0.16$ in normal and $r_{lf} = 0.82 \pm 0.08$ in acid-receiving groups regardless of the different groups. The individual values lie in all three groups on an unbroken straight line, and higher fat-weights are found only in the ascorbic and gluconic groups.

Surveying the effects of the prolonged administration of hexuronic acids, we can divide them in two groups. One is the same in the case of both acids, and the other is the specific effect of ascorbic acid. The first must be caused by some identical part common in both molecules, whereas the other is caused by the anti-scorbutic group present only in ascorbic acid. The effects in the first group are the following: Obesity of part of the animals, increase in liver weight and decrease of liver glycogen, the small increase of the fat-free body weight in some animals, the increase of the thymus may be attributed to the acidaemia caused by both acids. The specific effect, caused only by ascorbic acid, was the great increase in the weight of the spleen. It is to be noted that the spleen increases not only by over-dosage of vitamin C but also by its lack (DAL LAGO 1941). Extirpation of the spleen renders the symptoms of scorbut more severe (CHAHOVITCH and FRÁJND (1939), and also quickens the development of scorbut on a partly vitamin C deficient diet.

SUMMARY.

1. The effects of the administration from the beginning of growth to the end of it of 20 mg l-ascorbic or d-gluconic acid can be divided into two groups. One is common in both cases and is therefore caused by some identical radical in the molecules, p. e. their acidity. The other can be observed only in the case of ascorbic acid administration and is therefore caused by the specific anti-scorbutic group of this molecule.

2. The following effects are caused by the acidity of the two molecules: 11.5% greater body weight, 8% greater weight of the fat-free body, 25% increase in the connective fat, and 30% increase of the thymus. The liver, heart, kidneys, suprarenals and thyroids take part equally (with high correlation coefficients) in the increase of the body weight.

3. 40% decrease in the amount of liver glycogen is caused by the acidity of hexuronic acids. The glycogen and protein content of the muscle, the fat and protein content of the liver remain unaltered. The fat content of the muscles shows an increase of 20%. (The fat is probably deposited in the connective cells of the muscles.)

4. The effects observed only in the ascorbic acid-receiving group are: an increase of 100% in the weight of the spleen and an increase of 25% in the ascorbic acid content of liver and suprarenals.

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REFERENCES.

d'Alessandria and Haas H. T. A. (1943). Vitamine u. Hormone **3.** 168.
Backman, G. (1931). Erg. Physiol. **33.** 904.
Beznák A. B. L., Beznák M., Gáspár-Rády Zs. (1943). Ber. Physiol. Pharm. **131.** 237.
Beznák A. B. L., Beznák M., Gáspár-Rády Zs. (1947). *These Arch.* **17.**
Chahovitch X., K. Frajnd. (1941). Acta Path. (Beograd). 3. 98. 1939. Ber. Physiol. **125.** 523.
Dal Lago, Mario. (1941). Boll. Soc. ital. sperm. Biol. **16.** 59. Ber. Physiol. **127.** 263. 1942.
Elias H. (1912). Biochem. Z. **48.** 120.
Elias H., Kolbe L. (1913). Ibid. **52.** 331.
Fazekas I. Gy. (1939). Magy. Orv. Arch. **40.** 256.
Good C. A., Kramer H., Somogyi M. (1935). J. Biol. Chem. **100.** 485.
Kusimoto and M. Nazayasu. (1936). Jap. path. Soc. **26.** 153.
McClendon J. F., v.. Meysenburg L., G. J. Engstrand, F. King. (1919). J. Biol. Chem **38.** 539.
Mosonyi J. (1934). Hoppe-Seylers Zeitschr. **230.** 240.
Sheppard M., McHenry. G. W. (1939). Biochem. J. **33.** 656.

IMPROVING THE BIOLOGICAL VALUE OF BREAD MADE WITH CORN (MAIZE) FLOUR, BY THE ADDITION OF EXTRACTED SUNFLOWER MEAL.

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With 1 Figure and 4 Tables in the Text.
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The addition of corn (maize) flour to wheat bread diminishes its biological value. The diminished value is conspicuous principally from the following standpoints (BEZNÁK 1941):

a) Less amino acid. In wheat there is relatively little tryptophane and lysine (SÓS J. 1942); in zein, the chief protein of corn (maize), tryptophane is entirely lacking.

b) In wheat there is about five times as much nicotinic acid amide as in corn (maize), from which it follows that in corn bread the nicotinic acid content would be decidedly reduced.

c) In corn (maize) there is less mineral substance, notably calcium, than in wheat.

Otherwise in respect to calories corn (maize) bread is about equal to wheat bread. The above reduced values have principally a nutrition biological significance when a people's nourishment is in any case unvarying and incomplete, as in the case of difficult provisionment for city populations in war-time, or in provinces where the consumption of corn (maize) is constant and invaried.

Exception can be taken to the regular consumption of corn (maize) bread from a biological standpoint.

Numerous attempts have been made to improve the biological value of corn bread with the materials at hand in difficult circumstances. These experiments were unsuccessful, either because the materials recommended were still less nourishing than corn itself (as for example millet) (Z. MARKUDZE 1937, BECKER J. 1942) or because the material in

question had a taste disagreeable in bread, as for instance lucerne-flour (see below).

In what follows I report on experiments in the course of which I tried to improve the biological value of corn bread by the addition of extracted sunflower meal. The work falls into three divisions: 1) observations of its appetizingness for human beings; 2.) biological determinations in the growth of white mice; and 3.) chemical analyses.

I.

Determinations in Appetising value.

The observations were made on a group of 30 human adults in the following way: The persons taking part in the experiment received

TABLE I.

Appetising qualities of breads of different ingredients, on the basis of experiments conducted on 30 adult humans. The breads are listed in order of their diminishingly appetising qualities.

List	Ingredients p c.								
	Wheat	Rye	Corn	Barley	Sunfl.	Lucerne	Soya	Yeast	Remarks
1	35	10	35	10	10	—	—	—	20 % potatoes
2	45	—	25	10	20	—	—	—	"
3	30	10	30	10	10	2	6	2	"
4	50	10	20	—	20	—	—	—	—
5	40*	10	20	10	10	2	6	2	
6	30*	10	30	10	15	—	5	—	
7	35	10	20	10	15	4	5	1	"
8	20	10	35	15	20	—	—	—	"
9	20*	10	35	15	10	2	5	3	
10	50	10	20		10	10	—	—	

* whole wheat flour

at a time 10 sorts of bread — the ingredients of which were unknown to them — in slices, 24 hours after the baking. Each kind of bread was then classified according to the 30 decisions as to its appearance and taste. The results of this classification are given in Table I. In this Table we give the ingredients of the different breads and assemble them in accordance with their diminishing appetising qualities.

From the Table it appears that 20% sunflower meal can be added to the bread without appreciably affecting the usual taste. The reason for this is that sunflower meal is nearly tasteless. It was different in the case of lucerne flour, 2 % of which affected the appetising quality

of the bread, 4% of which in the bread brought it to 7th place, and 10 % to the last place on the list. Those taking part in the experiment in fact unanimously declared this latter bread uneatable.

II.

The Biological Experiments.

The biological experiments were conducted on 55 young mice. At the beginning of the experiment the weight of the animals was 8.2/ (± 2.1) g. The mice were put in 5 equal groups. The groups consumed the following foods:

Control group: barley-oats mixture +, for each mouse, 2—2 g.

TABLE II.

Ingredients of breads used in experiments on animals.

The animal groups	Ingredients				
	Wheat g	Corn g	Sunflower g	Potato mash g	NaCl g
I.	2500			600	50
II.		2500		600	50
III.	1250	1250		600	50
IV.	1250	750	500	600	50

of the following mash, cooked fresh each day: 1 part semolina-salt mixture (10 % wheat, 10 % rye, 50% corn (maize), 10 % barley, 50 % soya meal, 5 % casein, 5 % dried yeast, 5 % McCallum-Simmond's salt mixture) + 1.5 parts milk.

The animals of groups I—IV each received bread of different ingredients in a dry state, and as much water as they desired.

The ingredients of the breads investigated in the experiment are shown in Table II.

We measured the mice twice a week and registered the weights. For 30 days, in the tranquillity of their cages, the mice ate the above foods. Beznák and co-workers (1943) have shown that there are some diets on which animals as long as they are in a resting state, show a relatively good growth curve which diminishes when they are made to work, showing that the diet is not complete. Therefore, after these thirty days of quiet, our mice were made to work, in such a way that

at first they ran for 10 minutes, afterwards for 15 minutes a day, at a speed of 7.85 km/hr in a revolving drum.

Figure 1. shows the growth curve of the different groups of animals. The shaded columns on the abscissa represent the time of the running. From the Figure the following can be seen:

The average weight of the group of animals fed on pure corn (maize) bread, after an increase at the beginning lasting 20 days,

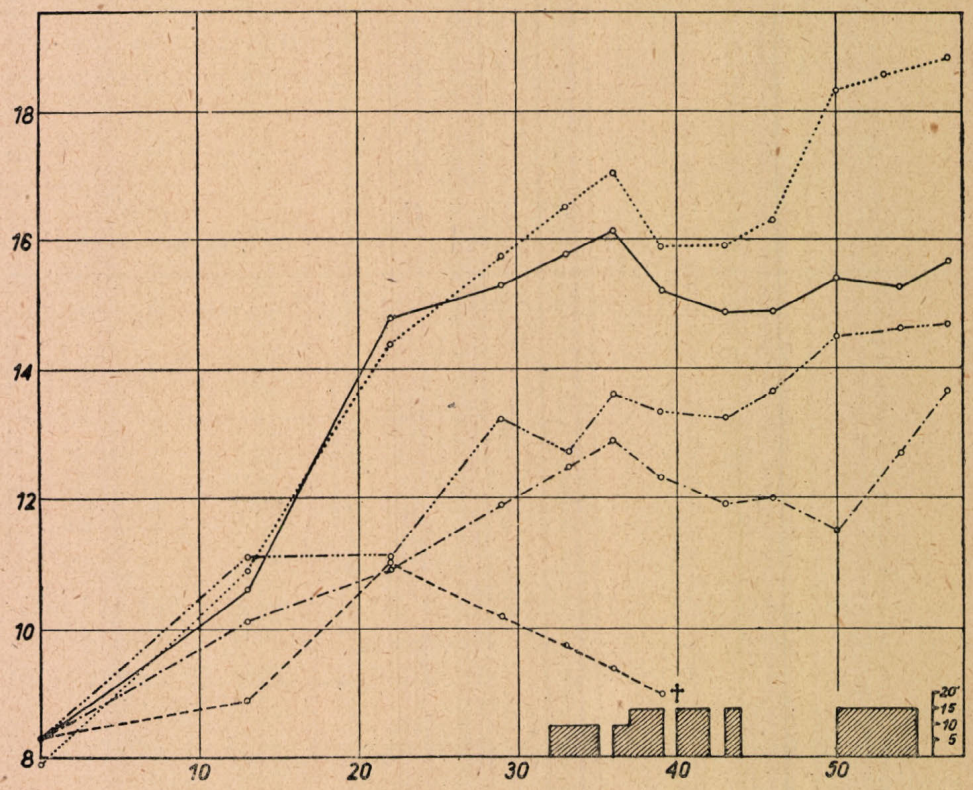

Figure 1. Growth curves of mice consuming different diets. Ordinate: average of weights in grams. Abscissa: time in days.

○.....○ Control group.

○———○ Group consuming bread No. IV (50% wheat flour, 30% corn flour, 20% sunflower meal).

○—...—...○ Group consuming bread No I (wheat flour).

○·—·—·—·○ Group consuming bread No III (50% corn).

○— — — —○ Group consuming bread No II (corn flour). Columns on the abscissa: running in minutes.

began to fall. The running further diminished their weight, and the animals died off, so that by the 8th day of the running period the whole group was dead. Their average body weight when they died was about the same as at the beginning of the experiment.

On pure wheat flour the mice grew, but their speed of growth was slow. During the running, after a slight loss in weight, growth continued at a tranquil, slow pace. By the 60th day of the experiment the growth curve had not yet reached the upper assymptote.

If we substituted corn (maize) for half the amount of wheat in the bread, the animals' growth remained somewhat less than that of animals fed on pure wheat-bread; the mixture was not entirely incomplete, as the growth curve — though low — after a passing drop when the work began, again mounted.

The growth of the group of animals receiving the bread containing 20% sunflower meal, for the first 35 days of the experiment ran almost parallel to the ideal growth of animals consuming a balanced diet. The growth curve was much more precipitous than that of the animals on any of the other breads, showing that the addition of 20% sunflower meal to corn bread is capable of increasing its biological value to a great extent.

During the running the situation changed. The body weight of both groups (control and sunflower) temporarily diminished. But while the mice of the control group, on a balanced diet, afterwards continued to grow at the same speed during the running period, the growth of the animals fed on sunflower meal bread definitely slowed up.

It appears from these experiments that the low biological value of corn bread can be improved by the addition of 20 % sunflower meal, so that it is superior to that of pure wheat bread.

III.

Chemical Analyses.

In the course of the analyses we investigated the following:

The contents of the different kinds of bread in dry materials, crude protein, ash and lime. The sunflower meal was thoroughly analyzed. Besides the above mentioned, we determined the sulphur content of the ash, the tyrosine and tryptophane in the amino acids, as well as the thiamine and the nicotinic acid amide.

METHODS.

a) Dry materials were determined in the usual manner, drying at 110°.

b) Crude protein: total nitrogen (according to KJELDAHL) multiplied by 6.25.

c) Ash: the materials investigated were incinerated in an electric furnace to constant weight.

d) Ca content: The ash got by incineration was dissolved in boiling dilute hydrochloric acid, was filtered, the filtrate neutralized with strong ammonium hydroxide, then again acidified with a few drops of glacial acetic acid. The lime in the solution was determined according to MURER's (1937) method.

e) Sulphur content: The filtered hydrochloric acid solution of the ash was treated with a 1% $BaCl_2$ solution and the resulting $BaSO_4$, after filtering, was burnt and measured.

f) We made the tyrosine and tryptophane determinations in the complete sunflower meal by hydrolyzing with 15% NaOH. The hydrolysis took place in an autoclave at a pressure of 3 atm. for 3 hours (D. DESEÖ 1934). In the hydrolysate the tyrosine was determined according to FOLIN's (1927, 1929) method, by BÁLINT's (1938) modification, the tryptophane after VIRTANEN and LAINE (1936) according to WINKLER (1934) with Stufenphotometer.

g) We applied the thiocrome method by RITSERT (1937) to determine the thiamine.

h) The nicotinic acid amide was determined as follows: The carefully measured air-dried material was boiled for 2 hours in a water bath with 10 times its quantity of n/50 H_2SO_4. The mixture was centrifuged, the precipitate twice again similarly extracted with 5 times as much n/50 sulphuric acid. The combined extract was neutralized with saturated Ba $(OH)_2$, again filled to known capacity and filtered. To 20 ml of the filtrate we added 5 ml M/5 KH_2PO_4 solution and put the reaction mixture in a water bath for 5 minutes, at 75—80°. Then we added 1 ml of fresh-made 5% Cyanogen Bromide solution to it and put it into the same water bath for 5 minutes. (E. BANDIER 1939). After cooling we added 1 ml saturated water aniline solution ,and after about 10 minutes we analysed the developed colour by a Stufenphotomer with S 43. 4/2.0 colour filter (ABDOH u. TÄUFEL 1942). In the control tube was 20 ml of the material examined with a solution of 5 ml hydrophosphate and 2 ml water. In the other control tube we

measured 20 ml distilled water with 5 ml hydrophosphate, 1 ml Cyanogen Bromide solution and 1 ml analine solution. Both control tubes were treated in the same way as the test. From the extinction of the test (E^r) were subtracted the values of the 2 controls (E^c1 and E^c2). The nicotinic acid amide content was calculated by a calibrated curve from the real extinction, $E = E^r - (E^c1 + E^c2)$.

In *Table III* we give some of the more important nutrition biolog-

Table III

Some nutritional factors occurring in extracted sunflower meal, in 85% wheat flour and in corn flour.

	Sunflower meal	Wheat flour	Corn flour
Protein g%	30[1]	8.6[3]	10.0[4]
Fat g%		1.5[3]	2.8[4]
Ash g%	6.4[1]	1.8[4]	0.86[4]
Ca mg%	430.0[1]	24.5[3]	16.0[5]
S mg%	5.7[1]		
Tyrosine mg%	1200.0[1]	900.0[1]	890.0[1]
Tryptophane mg%	250.0[1]	100.0[4]	60.0[6]
Lysine mg%	5.40.0[2]	14.0[4]	166.0[4]
Histidine mg%	4290.0[2]	214.0[4]	97.0[4]
Arginine mg%	2730.0[2]	273.0[4]	280.0[4]
Proline mg%	1580.0[2]	814.0[4]	518.0[4]
Nicotinic acid amide mg%	41.5[1]	5.3[4]	1.2[2]
Thiamine μg%	200.0[1]	110.0[4]	500.0[4]

[1] own investigations.
[2] A. W. Blagowestschenski and T. A. Schubert: Bioch. Z. 269, 375, 1934.
[3] R. A. McCance and E. M. Widdowson: The Chemical composition of Foods. London 1946.
[4] Sós J.: Magyar Néptalálkozástan. Budapest 1942.
[5] H. C. Sherman: Chemistry of Food and Nutrition New-York 1938.
[6] T. Ide: Zeitschr. f. d. ges. exp. Med. 24, 166, 1924.

ical data of sunflower meal, partly its own analyses, partly on the basis of literary data. For comparison we give the similar data for wheat and corn (maize) flour on the basis of the literature.

From the *Table* it appears that in the extracted sunflower meal

the very materials exist which in wheat or corn are present only in small quantities or not at all, primarily some amino acids (tyrosine, tryptophane. lysine), calcium and nicotinic acid amide. Thiamine was to be found in relatively small concentration in the extracted sunflower meal. The cause of this may be the fact that before the extraction the sunflower seeds were roasted at a high temperature, possibly destroying a greater amount of thiamine which may have been present in the raw seeds.

Table IV. shows the analysis of the different compounds of the breads examined.

From this Table too it appears that the sunflower can supply the lacks in pure wheat, or wheat-corn mixtures. It is interesting that sun-

TABLE IV.

Some nutritional factors occurring in the kinds of bread examined.

Kind of bread*	Water g %	Crude protein g %	Ash g %	Lime mg %
I.	48·1	9.08	1.3	39.8
II.	45.9	5.88	0.9	19.0
III.	48.1	7.62	1.2	28.8
IV.	52.1	9.75	1.4	68.0

* for the ingredients of the different breads see Table II.

flower provides greater water-binding capacity than wheat or corn. There is consequently more water in sunflower bread than in wheat or corn (maize) bread.

Discussion.

From Figure 1 it clearly appears that if we add a quantity of 20 % extracted sunflower meal to wheat-corn bread, its biological value is improved. The mice fed with bread prepared from sunflower meal grew very nearly as well as those on a complete balanced diet. The growth curves also show that the corn bread containing sunflower meal has more value than the bread prepared from pure wheat flour.

The growth of the group of mice fed on bread containing sunflower meal is principally conspicuous during the first period, in the resting state. In this phase of the experiment the sunflower bread seems an entirely adequate diet for the growing mice. But it can be seen that while the sunflower exceeds the wheat and the corn (maize) in biological value it still cannot make the wheat-corn ingredients com-

pletely adequate in the proportions investigated. The second period of the experiment showed this, when the animals undertook physical work. In the beginning, as effect of the work, the body weight of some groups of animals diminished. But while the control mice, on a balanced diet, after the beginning drop in weight quickly regained and again grew at a rapid pace, the growth curve of the mice fed on the bread containing 20% sunflower meal flattened out. Similar effects were observed by BEZNÁK, HAJDU and BEZNÁK (1934) in the case of rats fed on bread mixed with corn-cob flour.

The growth curve otherwise conspicuously showed the pure corn (maize) bread to be incomplete and confirmed the already known fact that an admixture of corn flour to wheat bread diminishes its biological value.

The chemical analyses show the cause for the sunflower meal's being able, in the relatively small quantity examined, decidedly to increase the biological value of the breads prepared from the different cereals. We found (Table IV) that the sunflower meal raises the bread's protein content. Pure wheat bread contains 9.08% crude protein. If we mix corn (maize) flour with it in a proportion of half, the protein content falls to 7.62%. But corn bread containing 20% sunflower meal has 7.5% protein, that is, 7.4% more than the wheat, or 30 % more than the corn bread. Sunflower meal contains, in significant concentration, those amino acids which are lacking in wheat and corn (maize), or occur only in very small quantities (Table III). Thus the sunflower meal not only increases the absolute quantity of nitrogen but also changes the bread's amino acid compounds in a biologically more balanced way.

We remarked in the introduction that corn (maize) has less worth than wheat, principally from the 3 standpoints of: protein amino acid compounds, less mineral substance (principally calcium) and finally because of its inferior nicotinic acid amide content. The lime content of the extracted sunflower meal is really high: 430 mg %; while in wheat bread we find 30.8 mg % calcium, which diminishes to 28.3 mg % when corn (maize) flour is added. We could determine 68.0 mg % Ca ions in the sunflower meal bread, which is higher than in the wheat bread by 72 %, and in the corn bread by 140 %. The sunflower meal is also richer in other minerals than lime and thus the total ash content is higher than that of either wheat or wheat-corn bread.

The low nicotinic acid amide content of corn (maize) bread can be improved by adding sunflower meal. Sunflower meal contains almost as much nicotinic acid amide as dried yeast. This circumstance is significant. It is also known that an incomplete corn meal diet causes pellagra in humans. The cause of this illness is primarily nicotinic acid amide deficiency, but it would seem that the lack of certain amino acids also have a role in its development (Beznák 1941). A small quantity of sunflower meal contains relatively much nicotinic acid amide, lysine and tryptophane, so that with really little sunflower meal added to the diet the occurrence of pellagra could be prevented. But attention must be called to the circumstance that no matter what value a food may have from a biological standpoint, we cannot think of introducing it into a people's diet if the taste is not agreeable, or if from it a food cannot be prepared which meets with the average popular taste. In the first part of the experiment the observations described show that sunflower bread seemed to suit this average popular taste. The taste of sunflower meal itself is neutral and so the taste of the food prepared from it, bread primarily, is not affected.

In sunflower meal, as in corn (maize) flour, there is no gluten. That is why it cannot be added to bread in optional proportions. In the preparation of such foods as are prepared from pure corn meal it can be used, or admixed, in the same way as corn meal or flour. We can put as much of it in bread as experience shows corn flour to be suitable, as much as will give us a well-baked bread. This amount is about 20—30%.

Summary.

1. The observations made on 30 adult humans showed that extracted sunflower meal in a proportion of 20% can be added to wheat flour, or to a mixture of wheat and corn (maize) flours, without spoiling the appetising quality of the bread made from it.

2. Young mice grow the best on a diet of wheat-corn (maize) bread containing 20% sunflower meal, less well on pure wheat, still less well on bread made of wheat-corn (maize) flour. On pure corn (maize) flour bread the animals did not grow and died.

3. The extracted sunflower meal contains such quantities of lime, tyrosine, tryptophane, lysine and nicotinic acid amide that by adding 20% of it to corn (maide) bread its low biological value is improved to such an extent that it is higher than that of pure wheat bread.

4. On the basis of these experiments the introduction of regular

consumption of sunflower meal is recommended in popular diets, principally in regions where pellagra is of common occurrence, and in times of distress when, because of lack of grain, bread is prepared with wheat flour mixed with corn (maize) flour.

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REFERENCES.

ABDOH, J. u. K. TÄUFEL. (1942). Z. *Untersuch. Lebensmitt.* **84,** 385 (Ber. ges. Physiol. 132, 175.) — Z. Untersuch Lebensmitt. **84,** 481. (Ber. ges. Physiol. **132,** 365.)
BÁLINT, P. (1938). *Bioch. Zeitschr.* **299,** 133.
BANDIER, E. (1939). *Bioch. J.* **33,** 1788.
„ and J. HALD. (1939). *Bioch. J.* **33,** 264.
BECKER J. (1942). *Mezőg. kut.* **15,** 16.
BEZNÁK, A. (1941). *Orvosi Hetilap.* **85,** 97.
BEZNÁK, M. v., I. HAJDU u. A. v. BEZNÁK. (1943). *Die Ernährung.* **8,** 33.
DESEÖ, D. v. (1934). *Bioch. Zeitschr.* **271,** 142.
FOLIN, O. and V. CIOCALTEU. (1927). *J. Biol. Chem.* **73,** 627.
„ and A. D. MARENZI. (1929). *J. Biol. Chem.* **83,** 89.
MARKUDZE, Z. (1937). *Bioch. J.* **31,** 1973.
MURER, H. K. (1937). *Ind. a Eng. Chem.* **9,** 27.
RITSERT. (1938). *Klin. Wschr.* 1397.
SÓS, J. (1942). *Magyar Néptáplálkozástan. Budapest.*
VIRTANEN, A. J. and L. LAINE. (1936). *Bioch. J.* **30,** 1509.
WINKLER. (1934). *Hoppe S. Zeitschr.* **228, 50.**

HAEMOPHYLBLUTGERINNUNG UND PLASMAKININ.

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(Mit einer Abbildung im Text.)

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Die Erscheinung der Haemophylie wurde bis in unsere Zeit auf sehr verschiedene Weise erklärt. Im allgemeinen stehen die Auffassungen in schröffem Gegensatz zueinander, die meisten aber glauben die Thrombocyten für die Verspätung der Gerinnung verantwortlich machen zu dürfen. Nach dieser Auffassung soll eine Erhöhung der Resistenz auftreten, wodurch die Abgabe der Thrombokinase eine Störung erfährt.

Vor kurzem gelang es L a k i in der Phase I. der Blutgerinnung das Auftreten eines kinaseähnlichen Stoffes (Plasmakinin) und damit zusammenhängend die autokatalytische Bildung des Thrombins nachzuweisen. (Laki 1943.) Nach L a k i werden die Thrombinbildung, bzw. die Blutgerinnung durch die Thrombokinase lediglich in Gang gesetzt, für die autokatalytische Thrombinbildung ist dagegen das kinaseähnliche Plasmakinin verantwortlich, das gleichzeitig mit der Thrombinbildung entsteht oder aktiviert wird. Nach Untersuchungen von L a k i wurde weiterhin festgestellt, dass die Verzögerung der Gerinnung des Haemophylblutes auf den Wegfall der autokatalytischen Thrombinbildung zurückzuführen sei (Laki, 1944), allein die Frage blieb offen, ob unter dem Plasmakininmangel ein Fehlen der Prokinase, oder des aktivierenden Faktors zu verstehen sei.

Um dieses Problem zu entscheiden, haben wir die nachstehende Versuche ausgeführt.

Das Prinzip des Plasmakinasenachweises ist folgendes. Die Wirkung des aus dem Gerinnsel gewonnenen Pressaftes wird auf unrecalzinierten, bzw. recalzinierten Plasmen festgestellt. In ersten Falle kann

nur in dem Pressaft anwesendes Thrombin, im zweiten auch nebst Thrombin entstehende Kinase zur Geltung gelangen.

Bildung der Plasmakinase im Blute.

Versuchsanordnung:

0,5 ccm Rinderoxalatplasma
0,5 „ 0,9% NaCl-Lösung
0,5 „ m/40 $CaCl_2$-Lösung .....1.)
Gerinnungszeit cca 1 Min.

Die Aktivität des aus dem Gerinnsel gewonnenen Pressaftes wurde nach verschiedenen Incubationszeiten in folgenden beiden Testen festgestellt:

1.) Recalzinierter Test

0,2 ccm Oxalatplasma
0,15 „ 0,9% NaCl-Lösung
0,05 „ 1% $CaCl_2$-Lösung
0,1 „ Pressaft aus 1.) .....2.)

2.) Oxalat-Test:

0,2 ccm Oxalatplasma
0,2 „ 0,9% NaCl-Lösung
0,1 „ Pressaft aus 1.) .....3.)

Die aus den Gerinnungszeiten berechneten Aktivitätswerten sind in Abb 1. dargestellt. (Unter der Einheit der Aktivität wird diejenige Wirkungskraft verstanden, welche die Gerinnung des zu dem Test verwandten Plasma in 1 Minute bei einer Zimmertemperatur von 20° C hervorruft.)

Wie aus der Abb. 1. ersichtlich gibt der Oxalat-Test (Kurve 1.) niedrigere Werte als der recalzinierte Test, (Kurve 2.), da in ersterem nur das Thrombin, in letzterem aber auch die vorhandende Kinase ihre Wirkung ausübt. Die Differenz der beiden Kurven (Kurve 3.) ergibt also die Plasmakinasewirkung.

Untersuchungen mit Haemophylblut.

Um zu entscheiden, ob die Verspätung der Gerinnnug des Haemophylblutes auf den Mangel an Prokinase zurückzuführen sei, wurden folgenden Versuche angestellt:

Zunächst untersuchten wir die Wirkung des Thrombins auf Nor-

mal-, bzw .Haemophylblut, sodann die Wirkung der Recalzinierung auf beide Blutarten.

Gerinnung mit Thrombin.

0,1 ccm Normal Oxalatblut (Mensch)
0,2 „ 0,9% NaCl-Lösung
0,1 „ Thrombinlösung . . . . 4.)
Gerinnungszeit: 34 Sek.

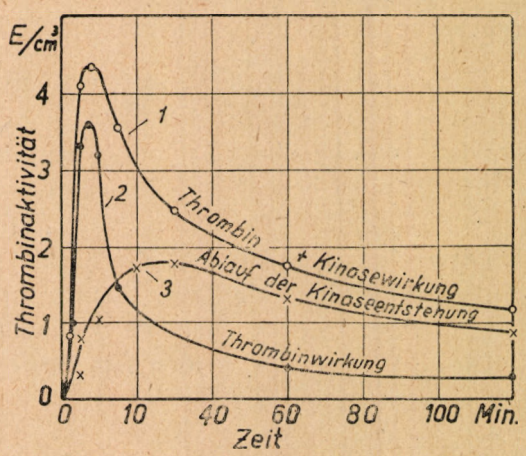
0,1 ccm Haemophyl Oxalatblut
0,2 „ 0,9% NaCl-Lösung
0,1 „ Thrombinlösung . . . . 5.)
Gerinnungszeit: 30 Sek.

Ergebnis: Mit Thrombin gerinnt Haemophylblut in gleicher weise wie Normalblut.

Gerinnungszeit mit Recalzinierung:

0,1 ccm Normal Oxalatblut (Mensch)
0,2 „ 0,9% NaCl-Lösung
0,1 „ m/40 $CaCl_2$-Lösung . . 6.)
Gerinnungszeit: 4 Min. 10 Sek.

0,1 ccm Haemophyl Oxalatblut
0,2 „ 0,9% NaCl-Lösung
0,1 „ m/40 $CaCl_2$-Lösung . . 7.)
Gerinnungszeit: 20 Min.



Ergebnis: Recalziniertes Haemophylblut gerinnt wesentlich schlechter als Normalblut.

Angenommen, dass die Gerinnungszeit des recalzinierten Blutes aus dem Grunde eine Verlängerung erfährt, weil zufolge Plasmakinin-

mangels die autokatalytische Thrombinbildung in Wegfall kommt, versuchten wir ob im Haemophylblut die Plasmakinase nachzuweisen wäre.

In parallellen Versuchsserien ist es möglich, die an Normal-, bzw. Haemophylblut erzielten Ergebnisse miteinander zu vergleichen.

0,5 ccm Normal Oxalatblut	0,5 ccm Haemophyl Oxalatblut
0,2 „ Thrombin cca 3 E/ccm 8.)	0,2 „ Thrombin cca 3 E/ccm 9.)
Gerinnungszeit: 20 Sek.	Gerinnungszeit: 20 Sek.

5. Min. nach Gerinnung wurde das Gerinnsel zerquetscht und ausgepresst.

a) Ohne Recalzinierung.

0,1 ccm Normal Oxalatblut	0,1 ccm Haemophyl Oxalatblut
0,2 „ 0,9% NaCl-Lösung	0,2 „ 0,9% NaCl-Lösung
0,1 „ Pressaft 8.) . . . . . 10.)	0,1 „ Pressaft 9.) . . . . . 11.)
Gerinnungszeit: 16 Min.	Gerinnungszeit 17 Min.

b) Recalziniert.

0,4 ccm Pressaft 8.)	0,4 ccm Pressaft 9.)
0,4 „ m/40 $CaCl_2$ . . . . . 12.)	0,4 „ m/40 $CaCl_2$ . . . . . 13.)

1 Min nach Recalzinierung wurde je eine Probe entnommen und die Wirkung auf recalziniertes Oxalatblut festgestellt:

0,1 ccm Normal Oxalatblut	0,1 ccm Normal Oxalatblut
0,1 „ 0,9% NaCl-Lösung	0,1 „ 0,9% NaCl-Lösung
0,1 „ m/40 $CaCl_2$	0,1 „ m/40 $CaCl_2$
0,1 „ recalz. Pressaft 12.) . 14.)	0,1 „ recalz. Pressaft 13 . . 15.)
Gerinnungszeit: 35 Sek.	Gerinnungszeit: 28 Sek.

Die Gerinnungswirkungen der Pressäfte beider Proben waren fast gleichen. Dies ist nur dann verständlich, wenn auch im Haemophylblut Kinase sich bildet.

Um die Möglichkeit einer Gerinnung durch Recalzinierung völlig auszuschalten, war es notwendig, die Wirkung der Pressäfte auch auf Haemophylblut zu untersuchen, indem recalziniertes Haemophylblut bestenfalls erst in 20 Min. zu gerinnen vermag. (S. Versuch 6.)

0,1 ccm Haemophylblut	0,1 ccm Haemophylblut
0,1 „ m/40 $CaCl_2$	0,1 „ 0,9% NaCl-Lösung
0,1 „ 0,9% NaCl-Lösung	0,1 „ m/40 $CaCl_2$
0,1 „ recalz. Pressaft 12.) . 16.)	0,1 „ recalz. Pressaft 13.) . 17.)
Gerinnungszeit: 30 Sek.	Gerinnungszeit: 30 Sek.

Die gleichen Gerinnungszeiten der Versuche 14, 15, 16, u. 17 beweisen, dass die in Normalblut nachgewiesene Proplasmakinase auch im Haemophylblut vorhanden ist, die durch das in den Test eingetragene fertige normale Thrombin aktiviert wurde. Dies bedeutet aber, dass die unregelmässige Gerinnung des Haemophylblutes nicht auf einem Proplasmakinasemangel beruht, sondern dass die Proplasmakinase im Haemophylblut zu Plasmakinase nicht aktiviert wird.

Die Rolle des Thrombins in der Aktivierung der Proplasmakinase.

Nach obigen Versuchen verfügt auch das Haemophylblut über Proplasmakinase. Trotzdem gerinnt das native Haemophylblut unregelmässig. Mann könnte also daran denken, dass das Thrombin des Haemophylblutes nicht imstande ist Proplasmakinase zu Plasmakinase zu aktivieren. Um diese Frage zu lösen, haben wir aus Haemophylblut durch Recalzinierung eine native Thrombinlösung hergestellt.

Mit dem so gewonnenen Pressaft als Thrombinlösung liessen wir Oxalatblut gerinnen um die Versuche 8.) u. 9.) zu reproduzieren.

0,4 ccm Norm. Oxalatblut	0,4 ccm Haemophyl Oxalatblut
0,2 „ 0,9% NaCl-Lösung	0,2 „ 0,9% NaCl-Lösung
0,4 „ m/40 $CaCl_2$ . . . . . 18.)	0,4 „ m/40 $CaCl_2$ . . . . . 19.)
Gerinnungszeit: 3 Min. 20 Sek.	Gerinnungszeit: 16 Min.

Beide Gerinnsel wurden nach 25 Min. zerquetscht. Die Pressäfte wurden als native Thrombinlösung verwendet, uzw. Normalblut mit Normal-Pressaft und Haemophylblut mit Haemophyl-Pressaft.

0,6 ccm Norm. Oxalatblut	0,6 ccm Haemophyl Oxalatblut
0,3 „ Pressaft 18.) . . . . . 20.)	0,3 „ Pressaft 19.) . . . . . 21.)
Gerinnungszeit: 2 Min. 40 Sec.	Gerinnungszeit: 3 Min. 20 Sec.

Die Gerinnung kann — wenigstens im Haemophylblut — nicht auf Recalzinierung zurückgeführt werden, weil das recalzinierte Haemophylblut — wie aus Versuch 19.) ersichtlich — erst nach 16 Min. gerinnt. Da im Versuch 19.) zweks Gerinnung kein fertiges Thrombin zugefügt wurde, so ist es klar, dass die in Versuch 21.) eingetretene Gerinnung ausschliesslich auf die Wirkung des während des Versuches entstandenen Haemophyl-Thrombins zurückzuführen ist.

Die nahezu gleichen Gerinnungszeiten in den Versuchen 20.) u.

21.) zeigen, dass in beiden Fällen in den Versuchen 18.) u. 19.) nahezu gleiche Thrombinmengen gebildet wurden.

Aus den Versuchen geht hervor, dass Haemophyl-Thrombin an Haemophylblut fast so schnell die Gerinnung bewirken könne, wie aus Normalblut entstandenes Thrombin an Normalblut. Es frägt sich nur, ob aus Haemophylblut auf die Wirkung des Thrombins hin Plasmakinase entstanden ist. Um dies zu entscheiden, übertrugen wir den Gerinnungswirkstoff des Gerinnsels auf neue Blutproben *a)* unrecalziniert, *b)* recalziniert.

Um die Gerinnungswirkung der Recalzinierung auszuschalten, werwandten wir hier Haemophylblut, wie in den Versuchen 16.) u. 17.)

a) Ohne Recalzinierung (Nur Thrombinwirkung).

Normalblut.	Haemophylblut.
0,1 ccm Normalblut	0,1 ccm Haemophylblut
0,2 „ 0,9% NaCl-Lösung	0,2 „ 0,9% NaCl-Lösung
0,1 „ Pressaft 20.) . . . . . 22.)	0,1 „ Pressaft 21.) . . . . . 23.)
Gerinnungszeit: 18 Min.	Gerinnungszeit: 16 Min.

Recalziniert:

0,2 ccm 20.) Pressaft	0,2 ccm 21.) Pressaft
0,2 „ m/40 $CaCl_2$ . . . . . 25.)	0,2 „ m/40 $CaCl_2$ . . . . . 24.)

b) 1 Min. nach Recalzinierung (Plasmakininwirkung).

0,1 ccm Haemophylblut	0,1 ccm Haemophylblut
0,1 „ 0,9% NaCl-Lösung	0,1 „ 0,9% NaCl-Lösung
0,2 „ recalz. Pressaft . . . 26.)	0,1 „ recalz. Pressaft . . . 27.)
Gerinnungszeit: 20 Sek.	Gerinnungszeit: 22 Min.

Die grossen Unterschiede in der Gerinnungszeiten zwischen 26.) u. 27.) beweisen, dass bei der Gerinnung mit Haemophyl-Thrombin keine Kinase entstanden ist, da in diesem Falle nach Recalzinierung — dem Kontrollversuche (26.)) ähnlich — eine wesentlich schnellere Gerinnung hätte eintreten müssen.

Während also auf die Wirkung des Normalblut-Thrombins oder auf die Wirkung einer seiner Faktoren die Kinasebildung vor sich gehen

kann, vermag dieser Prozess auf die Wirkung des Haemophylblut-Thrombins im Haemophylblut nicht einzutreten.

Die Ergebnisse unserer Untersuchungen sprechen dafür, dass im Haemophylblut die Proplasmakinase vorhanden sei, dass aber der aktivierende Faktor darin fehle.

LITERATUR.

K. LAKI: Studies. from. the Inst. of Med. Chem. Univ. Szeged. **3**, (1943). 5.
K. LAKI: Schweiz. Med. Wschr. 74, (1944). 13.

ANODIC OXIDATION OF CHOLESTEROL DERIVATIVES.

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(From the Hungarian Biological Research Institute, Tihany, Lake Balaton.)

With 1 Figure in the text.

(Received for publication 2nd September 1946.)

It is known that in the oxidative disintegration of certain derivatives of cholesterol, as well as in oxidation of the side chain, there are produced keto-alcohols and di-ketons belonging to the group of sexual hormones, or other substances valuable as starting products in the synthesis of sexual hormones.

It is also known that oxidation of the side chain takes place with the usual oxidative materials (chromic acid and potassium-permanganate).

In the following we describe a procedure in which the oxidation takes place electrolytically, i. e., with nascens oxygen, developed anodically.

Electrolytical oxidation as a method in preparative sterol chemistry is not extensively worked out. Its use is, however, desirable as it enables experimental conditions to be varied largely, and it seems likely that with its help oxidation, especially of large molecules, where the oxidation is not one-sided, could be studied more elaborately.

Naturally anodic oxidation of cholesterol derivatives is not one-sided. By the splitting off of the side chain other oxidative products (aldehydes, carbonic acids) besides ketons and keto-alcohols are produced. As the procedure we describe in the following is concerned with the production of ketons created by the splitting off of the side chain, a consideration of the other oxidative derivatives falls outside the scope of this work.

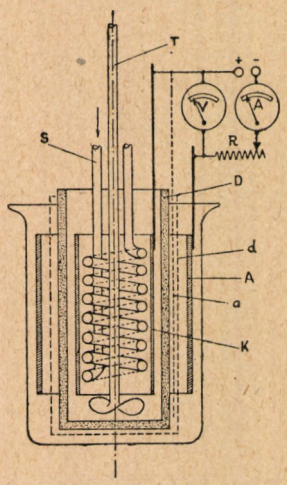
As regards practical use, according to the experiments so far performed, production does not exceed, indeed in some cases is less than, that of other methods. Therefore the experiments published here

should be considered chiefly as informative. They are the basis of work in progress, which is concerned with the study of the mechanism of oxidation.

During the course of the experiments saturated and unsaturated steroid ketons were produced.

From the saturated ketons we succeeded in preparing 3-acetoxi-etio-allo-cholanone and androsterone, the former from acetate of dihydrocholesterol the latter from acetate of epi-dihydrocholesterol, the isolation of both products in its semi-carbazone form.

From the unsaturated ketons, worthy of mention may be dehydro-androsterone, which can be made by the anodic oxidation of cholesterylacetate-dibromide.



The experimental set-up is shown in Figure 1.

The anolyte is put in the clay diaphragm (D) and the catholyte in the outer glass container.

The conduction of the cell is ensured by the introduction of an electrolyte which has great conduction capacity and is indifferent from the point of view of oxidation.

In some cases, especially during the oxidation of saturated sterols there is the disadvantage that the pores of the diaphragm (D) become clogged with the material we are oxidizing. This greatly increases the electric resistance of the cell and hinders long-lasting electrolysis, or makes it altogether impossible. In such cases it is desirable to surround

the diaphragm containing the anolyte with another electrolyte separated by a second diaphragm (d) of the same charge. The second diaphragm is immersed in the catholyte. The charge is supplied by a second electrode (a) connected to the anode. By transforming the cell a this way the clogging of the diaphragm generally does not occur.

The size of the electrodes (A and K) are determined by the size and charge of the cell.

The cell becomes very warm during the electrolysis. To avoid overheating, or to ensure an even temperature, we employ an inner cooling system.

The anolyte is stirred during electrolysis by a motor.

The electric charge of the cell is regulated by a resistance (R) and measured by an ampere-meter (A).

The potential difference between the electrodes is shown by a volt-meter (V).

From these potential differences we can determine the resistance of the cell. By changing the composition of anolyte and catholyte we generally succed in keeping the resistance low.

The Experiments.

Example 1.

50 g dihydro-cholesteryl-acetate is dissolved in 1500 ml glacial acetic acid, to which is added 150 ml 50% sodium acetate water solution. This mixture is poured into a 2 l clay diaphragm and is electrolysed as anolyte. The danger of the anolyte diffusing towards the cathode space is avoided by surrounding the diaphragm containing the anolyte with another positively charged electrolyte separated by a second diaphragm. The second diaphragm is immersed in the catholyte. In this second, outer diaphragm and in the cathode space the electrolyte employed consits of one weight of 50% sodium acetate water solution and 6 weights of glacial acetic acid.

The electrodes are made in the form of a hollow cylinder, the anode being made of a gold or platinum plate and the cathode of lead. As electrode into the second diaphragm a platinum wire, bent into the form of a circle, is used, the outlet of which is connected to the anode.

The density of the current, on the basis of the surface of the inner anode, is 80—100 milliamperes per cm^2. The temperature of the anolyte is kept around 80°C, by the aid of a cooling spiral of glass. The anolyte is vigorously stirred during electrolysis.

The amount of the current conducted through the system during electrolysis is 2.2 ampere-hours per 1 g of the starting substance. This means that, for example, in the present experiment where the surface of the anode was 150 cm^2 and the strength of the current 12 A, the time taken for the electrolysis was about 9 hours.

Having finished the electrolysis, the two anode solutions are united and condensed to about 300 ml in vacuum. The residue is poured into 2 l water and the resulting substances, forming an oily precipitate, are extracted with ether. This ether solution is washed with 1% NaOH and several times with water, then it is dried and evaporated.

The volatile oxidative products are expelled from the residue with water vapour and the remaining amorphous product is re-dissolved in ether. The ether solution is then dried and evaporated.

We get a light brown substance which contains oxidation products and some unchanged starting material. From this we prepare the semi-carbazone of the 3-acetoxy-etio-allo-cholanone formed during oxidation, by boiling it in alcoholic solution with semi-carbazide-acetate.

The melting point of this product after purification is 260—262°C. Mixture with the same substance differently produced causes no depression of the melting point.

Example 2.

The solution of 50 g epi-dihydro-cholesteryl-acetate in 1500 ml glacial acetic acid is electrolysed as in the first example.

The preparation of the substance produced is also carried out as above. Androsterone formed during the procedure is isolated as semi-carbazone. The semi-carbazone purified through re-crystallization does not depress the melting point if mixed with the same substance prepared by chromic acid oxidation.

Example 3.

50 g cholesteryl-acetate dibromide and 250 g water-free potassium acetate are dissolved in 2 l glacial acetic acid. The solution is poured into a clay diaphragm; the anode, which, can be a graphite electrode or a hollow platinum cylinder, is immersed in it. The diaphragm is put into the catholyte.

The catholyte consists of a 1:1 mixture of a 50% sodium acetate water solution and of glacial acetic acid. A lead cylinder is employed

as cathode. The density of the current is 80—100 milliamperes per cm^2 of anodic surface.

The temperature in the system increases during electrolysis. The temperature of the anolyte is kept between 80—90°C by cooling.

The starting material precipitating from the anolyte at the beginning gets oily during electrolysis and is later dissolved.

The amount of current employed is 1.6 A per 1 g cholesteryl-acetate dibromide. In the present case, where the surface of the anode was 200 cm^2 the strength of the current was 16 A, the time taken for the electrolysis was 5 hours.

After completing the electrolysis the anolyte is separated and, after adding 30 g zinc powder, is heated on a water bath. The solution is then filtered, the filtrate concentrated to one-fifth in vacuum. The residue is poured into 2 l water and resulting precipitate dissolved in ether. The ether solution is shaken several times with 5% NaOH to free the product of the carbonic acid produced during oxidation. The solution is then dried and evaporated on a water bath. Volatile substances are dispelled from the residue with water vapour and the remaining substance re-dissolved in ether. The solution is then dried and evaporated. 2 g semi-carbazide acetate dissolved in 25 ml alcohol are added to the residue, and the whole boiled on a water bath for some hours.

A precipitate consisting of colourless crystals is formed. This precipitate is washed several times with hot water after filtration. The substance is dehydro-androsterone semi-carbazone which melts after recrystallization from chloroform-methanol at 270—275°C.

To isolate dehydro-androsterone, 100 mg of the carbazone melting at 270—275°C are suspended in the mixture of 2 ml alcohol, 0.5 ml water and 0.1 ml concentrated sulphuric acid. The suspension is boiled on a water bath for one hour. The resulting solution, clear as water, is neutralized with a 20% solution of NaOH and, after the addition of further 0.5 ml alkali, warmed for another half hour over the water bath. The solution is filtered while hot and the filtrate is diluted in twice its volume of water. In cooling the dehydro-androsterone crystallizes in colourless silky needles.

The product is filtered, washed with a little 40% alcohol and dried. The substance thus obtained melts at 142°C. After re-crystallization from petrole-ether (b. p. 65—80°C) the melting point rises to 148°C.

Grateful acknowledgment is made to Mrs. J THOMPSON VASS for the English translation.

Remark:

Part of the experiments were carried out with V. BRUCKNER in the Organic Chemistry Institute of the University of Szeged, partly in the laboratories of the GEDEON RICHTER S. A. Pharmaceutical Mfg. Co.

See Hungarian Patents Nos 120106, 123574 and 123023.

PREPARATION OF 7-DEHYDROCHOLESTEROL, AND OF 7-OXYCHOLESTEROL.

By: ANDRÁS KRÁMLI.

From the Hungarian Biological Research Institute, Tihany, Lake Balaton.

(Received for publication 7th October 1946.)

7-dehydrocholesterol was first made by WINDAUS, LETTRÉ and SCHENCK (1935). Essentially their procedure was to disintegrate the dibenzoate of 7-oxycholesterol in a high vacuum at a temperature of 220°C. Then with the appearance of a molecule of benzoic acid, dehydration occurs between the carbon atoms Nos. 7 and 8 and, besides 1 molecule of benzoic acid, there results benzoate of 7-dehydrocholesterol, from which, after hydrolysis, 7-dehydrocholesterol is produced.

In the present work we speak of a process whereby the preparation of 7-dehydrocholesterol from 7-oxycholesterol takes place in one step. In the beginning we carried out the experiments with the usual methods of dehydration but they gave an unidentifiable resinous product. Good results were obtained with an oxalic acid water solution as dehydrolizing agent. Employing different concentrations of oxalic acid we found a 55—60% solution to be the most suitable. Use of a more dilute oxalic acid brought about a weaker production as judged by the rotatory power of the raw product. It is very difficult to isolate and purify the product because of the presence of subsidiary materials. Improvement in production and perfection in isolation are in process of experiment.

The starting material used, 7-oxycholesterol, was prepared by WINDAUS and co-workers from acetate of 7-ketocholesterol after MEERWEIN and PONNDORF's method with isopropylate of aluminium. In the present work the idea occurred of reducing 7-ketocholesterol in an alcoholic medium with alkali metals. As the reduction brought about by metallic sodium in the alcoholic medium results in an unidentifiable, dark product, experiments were carried out to slow down the speed

of the reaction with indifferent solvents. The best result was obtained by reduction in a mixture of dried benzene and methyl alcohol at room temperature. The reaction mixture then shows no darkening and the isolation of the product causes no difficulties.

The acetate of 7-ketocholesterol, which MAUTHNER and SUIDA (1896) first described, took place by anodic oxidation from cholesteryl acetate.

Details of experiment.

Preparation of 7-oxycholesterol.

2. g 7-ketocholesterol is dissolved in a mixture of 100 ml benzene and 180 ml abs. methyl alcohol. To this solution 16 g metallic sodium is very gradually added, while keeping it at a temperature of less than 20°C. When the sodium is entirely dissolved the mixture is poured into 500 ml water. Liquids in 2 layers result. The upper, benzene layer is separated, then washed with dilute solution of sodium hydroxide and finally several times with water. It is dried with water-free sodium sulphate and evaporated in vacuum. From the remaining light yellow oil the 7-oxycholesterol crystallises on rubbing with 50 ml petrole-ether, the resulting crystals are separated by filtration and recrystallized repeatedly in a mixture of benzene and hexan. A snow-white crystallized powder results with a melting point of 168—174°C, which heated with chloralhydrate gives a strong blue colour reaction, in a chloroform solution with concentrated sulphuric acid, a dark violet. When it is recrystallized with ethyl alcohol the melting point mounts to 176—178°C.

Remark: Acetate of 7-ketocholesterol can also be employed as starting material. The acetyl group splits off in the course of the reaction.

Preparation of 7-dehydrocholesterol.

10 g 7-oxycholesterol is dissolved in 50 ml boiling ethyl alcohol and the hot solution emulsified in 100 ml 60% oxalic acid water solution. From this emulsion the alcohol is evaporated over a water bath and the remaining mixture is boiled again until the resulting reaction-product condenses into drops of yellow oil, which takes place after about 1—1 1/2 hours of heating. The reaction mixture is cooled and the product, stiffened into a solid mass, taken up in ether. This ether

solution is washed with water, then several times with dilute solution of sodium hydroxide, then dried with water-free sodium sulphate, and evaporated. To eliminate the effect of oxidation by air the processes described are carried out in carbondioxide atmosphere.

The substance remaining after the evaporation of the ether solution is crystallized in a mixture of ether and methyl alcohol. A white substance results which, when exposed to air, turns lemon yellow, composed of small, flat crystals, strongly laevo-rotatory, which melts unsharp, with decomposition, giving up its water of crystallisation at between 100—130°C. The product dissolves well in ether, hexan and chloroform. It is difficult to dissolve in methyl alcohol and ethyl alcohol. Further purification takes place by repeated crystallization from ether-methyl alcohol, when the melting point rises to 140°C. Its chloroform solution with sulphuric acid and with chloralhydrate shows an intense violet-blue colouration.

Remark: Part of the experiments were carried out in the research laboratories of the Gedeon Richter, S. A., Pharmaceutical Mfg. Co. Budapest.

Grateful acknowledgment is made to Mrs. J. Thompson Vass for the English translation.

REFERENCES.

Gedeon Richter, S. A., Hungarian Pat. Nos. 128284 and 128285.
Mauthner and Suida. (1896). *Mh. Chem.* **17.** 593.
Meerwein and Ponndorf. (1926). *C.* I. 1893.
Windaus, A., Lettré, H. and Schenk, Fr. (1935). *Liebigs Annalen.* **520.** 98—106.
Windaus, A., Lettré, H. and Schenk, Fr. (1936). *C.* I. 355—356.

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