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NETHERLANDS CENTRAL INSTITUTE FOR BRAIN RESEARCH,
AMSTERDAM

PROGRESS REPORT 1969

DIVISION OF MORPHOLOGY

1. Section of Histology and Cytology

Prof. Dr. J. Ariëns Kappers:

Aid was given to Miss G. Mehring and Drs. H. Romijn concerning their work on, respectively, the light- and electron microscopic investigation of the pineal organ of *Testudo hermanni* and its innervation, and on the light- and electron microscopic investigation and innervation of the pineal organ of the rabbit.

Miss G. Mehring, guestworker from Germany:

A light- and electron microscopic investigation was performed of the pineal organ of the tortoise *Testudo hermanni*. The results have been offered for publication in the Zeitschrift für Zellforschung and will serve as a German doctorate thesis. Two main cell types could be distinguished in the pineal of this reptile: 1. secretory rudimentary photoreceptor elements, and 2. supporting elements of an ependymal nature. Both cell types have been described in detail. Like in lizards, the rudimentary photoreceptor cells show a rudimentary development of the outer segments. Their well-developed Golgi apparatus is involved in the production of dense-core vesicles of considerable size. The secretory products are released into large intercellular spaces which are connected with the pericapillary spaces. Fluorescence microscopy revealed that these dense-core vesicles probably contain serotonin as they do in the pineal of lizards. It appears that the epithelial sensory nerve cells are degenerating. The number of pinealo-fugal sensory fibers reaching the epithalamic region of the brain is small. Some of them reach the posterior commissure while other fibers run lateral to the subcommissural organ. The exact site or sites of their termination could not be determined. In the pineal stalk degenerating myelin sheaths belonging to the sensory pineal fibers could be demonstrated. Sympathetic nerve fibers, the terminals of which contain clear as well as dense-core vesicles, were shown to be present within the pineal epithelium. From all evidence available it appears that, also in this reptile, the direct photosensory pineal function is regressing while the secretory function of the organ develops. The fact that degenerating sensory nerve cells and degenerating myelin sheaths of pineal sensory fibers were shown points to a regression of the pineal photosensory function during later stages of ontogenetic development.

R. A. C. de Vries:

A new research program was started which concerns the mode of action of the pineal gland on the gonads of male rats prior to puberty.

Pilot studies are now being made in pinealectomized rats on changes in the activity of the enzyme thiamine diphosphate phosphohydrolase (TPP-ase) in any of the hypothalamic nuclei as revealed by histochemical techniques. In these rats, moreover, quantitative changes are studied in the content of gonadotropins in the cells of the anterior pituitary as shown by the staining method of Herlant.

2. Section of Comparative and Quantitative Neuroanatomy

Dr. G. J. Smit:

Investigations were continued on the rabbit cerebral cortex by the analysis of a second area (the precentral). Such analysis leads to a description of a number of anatomical parameters of the neurons as a function of depth below the pial surface. These parameters are: cell body volume, nuclear volume, number of first order dendrites, and the structure of the dendritic tree. The descriptions quantitatively characterize a given area by means of the pattern of increases and decreases of the neuronal parameters. These investigations led to a series of 7 papers prepared this year in collaboration with Dr. E. J. Colon, which are, apart of the first, still in press.

It was found that the only essential difference between both areas investigated, *i.e.*, the striate area and the precentral area of the cortex, is a difference in thickness. When for both areas the cortical thickness for each area is transformed to 2000 arbitrary units of length, the pattern of increases and decreases in the neuronal parameters is essentially the same. If such a finding were to apply to all the areas of the cerebral cortex in mammals, new conceptions about its structure and function would be the result. In order to investigate this point, the necessary additional histological material of the rabbit cerebral cortex has been prepared this year and the new investigations are now in progress.

Much time has also been devoted to statistical advice to other investigators with respect to the planning of their experiments and the analysis of their data. This activity is becoming more and more important, but only some examples will be mentioned here. In the first place, there are the investigations of hibernation in the golden hamster by Dr. J. H. Smit-Vis of the Anatomical Laboratory of the University of Amsterdam, together with whom Dr. Smit has published 2 papers on the relationship between hibernation and sexual activity. Secondly, the investigations of Dr. T. J. de Vlieger of the Biological Laboratory of the Free University, on the spontaneous activity of the isolated central nervous system in snails have been evaluated. Statistical analysis demonstrated the necessity of an improved experimental set up in which a number of nuisance variables

could be better controlled. Finally, a very intensive collaboration with Drs. A. P. van der Meché has led to the demonstration of interference of the "spontaneous" behaviour of the stickleback with its learning behaviour in a test situation. Therefore, the patterns caused by spontaneous behaviour are now being given closer attention as an important variable to be carefully monitored, and also since they appear to be very interesting in itself.

Drs. K. C. Hodde :

The study of the central nervous system of *Scylliorhinus canicula*, started in 1964, has been continued. The structure of the brainstem was studied in normal material. In addition, some experimental material, obtained during a stay in the laboratory of the Association of Marine Biology in Plymouth (G.B.) in 1966, was examined with respect to certain fiber systems, especially the ascending systems in the spinal cord, using the Nauta method. As a result of this pilot study, all the crucial technical problems could be solved. Consequently, new material was obtained during a second and third stay in Plymouth (April 3rd – June 5th; September 1st – October 15th 1969). Lesions were made at different levels in the spinal cord and medulla oblongata while also some of the dorsal spinal roots were severed.

Finally an electron microscopic study of the spinal cord and cerebellum was started which has given some preliminary interesting results.

Drs. P. Kenemans :

An investigation concerning the structure of the brain stem of the primitive bony fish *Polypterus* was continued and expanded.

From fresh material, well fixed due to a new perfusion technique, some new silver impregnated and Nissl-stained series were made. This was done not only for *Polypterus*, but also for the closely related *Calamoichthys*.

A quantitative analysis of the cranial motor nuclei was completed. The current study of the sensory nuclei, the cells of the reticular formation and the cerebellum, and also of the ascending and descending fiber systems is at an advanced stage.

An attempt is now being made to represent the most important results by means of a topological transformation into a two dimensional model, which, after the variation within the species has been established, will allow comparisons with other species.

R. T. Krediet :

Most of the research time was spent in studying the brain stem of the turtle *Testudo* and of *Polypterus ornatipinnis*, a primitive bony fish.

After an unsuccessful attempt to make a topological analysis of the longitudinal grooves in the brain stem of *Testudo*, a topological two-

dimensional projection was made of a Nissl-stained series of *Polypterus*, complementing the investigations on this species by Drs. P. Kenemans.

Comparisons were made between the results of both of these investigations, which enabled this new method to be critically evaluated.

Drs. C. J. van der Sloot :

Following a preliminary study about the termination of dorsal root fibers, an extensive investigation was started on the architectonics of the spinal cord of the turtle (*Testudo hermanni*).

Within this scope, special interest has been given to the large neurons of the dorsal horn. Two types can be distinguished, one of which has a multipolar perikaryon, thus resembling the motoneurons of the ventral horn, and the other looking more like spinal ganglion cells, *i.e.* bi- or pseudo-unipolar. Several histological techniques were tried out to demonstrate the cell processes in order to follow them through the dorsal root as suggested by earlier authors. It is also planned to study the central connections in order to compare especially the second type of neuron with the so-called intramedullary sensory cells which have been described for the spinal cord of fishes, amphibia and man (cells of Rohon-Beard).

Furthermore, various cell columns of the spinal cord have been studied also with histochemical techniques, in order to correlate them with the nuclei of the cranial nerves.

Drs. E. van der Wiele :

The study of the secondary olfactory connections in the turtle by Drs. E. van der Wiele has this year taken a new direction. It is now primarily concerned with a quantitative study of the olfactory system, as a first step and as a pilot-study a method has been developed for a quantitative analysis of the olfactory membrane. Much attention has been devoted to gain insight in the accurateness and limitations of the method.

By means of such an analysis it will be possible to find out whether the receptors are homogeneously distributed over the olfactory membrane or whether they show certain density gradients. Moreover, it will be possible to compare the olfactory membrane with those of Jacobson's organ and to see if there exist quantitative differences between both.

3. Section of Histochemistry and Cytochemistry

Dr. J. F. Jongkind :

The work in this department is centered around the cytochemistry of neurosecretory phenomena taking place in the mammalian hypothalamus.

The cytochemical methodology of Lowry has been applied to the question of the role of Thiamine-pyrophosphatase activity in the supraoptic nucleus of the rat.

Microdetermination of Thiamine pyrophosphate (cocarboxylase) and

determination of the activity of TPP-dependent enzymes such as transketolase and α -ketoglutarate dehydrogenase are still in the developmental stage. These microtechniques will be applied to dissected pieces of the activated supraoptic nucleus of the rat.

In connection with this investigation, substrate measurements have been done in dissected pieces of supraoptic nucleus. The problems due to deficient freezing of the decapitated heads will be published as a short communication in the J. of Neurochemistry.

A joint research together with Dr. M. A. Corner of the department of Comparative and Developmental Neurophysiology was performed. The aim of this cooperation is to study the biochemical events during ontogenesis. Substrate measurements have been done to study the energy reserve fluxes in the forebrain of decapitated chick embryos and to correlate these biochemical findings with the electrical activity of the chick brain before and after decapitation.

Drs. D. F. Swaab :

The neurosecretory activity of the supraoptic and paraventricular nuclei of the rat was followed by means of a semi-quantitative histochemical parameter (Jongkind and Swaab, *Histochemie* 11, 319, 1967) during pregnancy, parturition and lactation.

During the oestrous cycle, after gonadectomy and during light-induced persistent oestrous the neurosecretory activity is paralleled by the blood-level of gonadotropic hormones (Ramirez and McCann, 1964; McClintock and Schwartz, 1968; Gay and Midgley, 1969; Parlow, 1964; Negro-Vilar, 1968; Lawton and Schwartz, 1967). This relationship was confirmed by injection of estrogens, which decreased the neurosecretory activity and injection of gonadotropins which increased the neurosecretory activity.

4. Section of Electron Microscopy

Drs. H. J. Romijn :

Several techniques have been studied and developed during the first three months of this year, after the establishment of the section in the year before. At the end of March an investigation was started on the pineal gland of the rabbit, concerning:

- a. The general cellular structure. By means of the electron microscope interesting new data have already been obtained.
- b. The innervation. With the aid of light-microscopical techniques, *e.g.* silver staining, we hope soon to get an idea on the nerve fibers reaching the pineal gland: the sympathetic innervation from the superior cervical ganglia, and the course of the fibers running from the habenular and/or posterior commissures to the organ; the fine structure (possible synaptic contacts on the pinealocytes etc.) will be studied by electronmicroscopy.
- c. The endocrine activity. In view of the diurnal rhythm of biogenic

amines, lipids etc. in the pinealocytes, research upon this topic will be started by means of investigations of the cellular ultrastructure at different times of the day and after the application of drugs, using in addition autoradiographic techniques.

A. Smith, part-time worker:

An investigation was started on the topographical relationship between the pineal gland of the rabbit and the large veins surrounding the organ. In contrast to the rat pineal organ, most part of the pineal of the rabbit appears to be surrounded from all sides by blood vessels which makes experimental removal of the organ practically impossible.

DIVISION OF PHYSIOLOGY

1. Section of Experimental Neurology

Dr. J. P. Schadé :

In order to study the functional aspects of reduced oxygen and nutrient supply to the brain, preliminary experiments have been performed to investigate the usefulness of experimental arteriosclerosis as a model. For this purpose a diet was composed consisting of regular rabbit food containing 1% cholesterol, while in addition the electrolyte content was changed.

To analyse the sequelae of arteriosclerosis, screw electrodes were inserted into the skull for recording electrical brain activity. As far as could be judged from the histological analysis of a few preparations, arteriosclerotic degenerations can already be seen after 7 weeks.

Continuous EEG evoked potentials and arousal phenomena were recorded in free moving animals. All data were recorded on tape for quantitative computer analysis. After 3 months (December 1969 – January 1970) a large series of experiments will be performed in order to detect changes in the functional activity of neurons at a unit level.

Dr. J. P. Schadé and Ir. H. Smith :

As a basis for experimental work a system-analysis was performed using iterative data and some preliminary investigations of the hypothalamus.

The Hypothalamic Integration Neuron (HIN) is defined as a specialized unit which shows dual properties: (a) neurotransmitter sensitivity; both excitatory and inhibitory synaptic mechanisms have been demonstrated, and (b) sensitivity to environmental factors such as temperature, glucose, hormones. A hypothalamic integrating system consists of two sets of HIN's showing the same sensitivity properties, but as far as the environmental factor is concerned either + or – sensor property. One of the most striking findings is the fact that the + and – sensors are present in unequal numbers which indicates that the measuring system has a different scale either below or above the norm. We are apparently dealing with a

stochastic setpoint over a group of neurons which may be adjusted by neuronal synaptic mechanisms.

The characteristic properties of the HIN may be found in neurons and neurosecretory elements of the hypothalamus. The phenomenon of neurosecretion means, in this respect, that cells characterized by all morphological and physiological features (action potentials and synaptic mechanisms) as true neurons are able to produce substances that act as hormones or releasing or inhibiting factors. These substances are probably released in a quantitative manner by action potentials arriving at the transmitting part of the cell.

The HIN receives information by parallel input, in contrast to computing units which operate mainly by serial input. The receptive pole of the HIN is not only the seat of the interplay between the hundreds and thousands of excitatory and inhibitory synapses, but is also the receptor site of environmental stimuli.

Simulation of these and other properties of the HIN has been performed with the IBM 1130 Continuous System Modelling Program.

Dr. J. C. de Valois, Drs. J. P. C. Peperkamp and B. van Cranenburgh:

The isotope clearance method for the measurement of cerebral blood flow has been used to study the cerebral circulation of the rabbit under a variety of conditions. To get quantitative flow data, a Fortain IV computer program has been developed which enables to distinguish between a relatively fast perfused compartment and a slower perfused one. The cerebral blood flow of the rabbit under normal conditions as well as under 1/2% fluothane anesthesia are estimated. We found a decrease of 20% during fluothane anesthesia. In a series of 80 experiments the effect of 9 vasodilating drugs were tested upon the cerebral circulation, in a standardized way, in normal rabbits. The results of these measurements support the view that the cerebral vessels do not react to peripheral vasodilating drugs like papaverin or react with a distinct vasoconstriction as is the case with nicotinic acid. These observations might have some interesting implications in clinical neurology.

The obvious lack in autoregulation which occurred after the intracarotid injection of nicotinic acid goes along with a shift in relative weights of the fast and slow perfused compartments. The relative weight of the fast compartment decreases significantly. This phenomenon might give some insight in the intricate mechanisms of autoregulation.

Dr. J. L. Blom:

Using micro-electrode techniques, the activity of 342 neurons before, and of 180 neurons after administration of MSO were recorded in 40 rabbits divided in 4 groups. On 26 animals observations were done to investigate the differences occurring with various routes of administration and different doses.

After recording the neurons and registration of their position below the pial surface, interval histograms were made with a special purpose computer, comprising more than 95 per cent of all the first-order intervals. The mean firing frequency of the complete period of recording was calculated and the sequence in time of the action potentials analyzed. The reaction of the mean firing frequency and some parameters of the interval histogram with depth was analyzed with some statistical methods, as was the serial correlation of consecutive intervals. The interval histograms are described with their central moments and, by means of statistical and mathematical procedures, partly fitted to a gamma-distribution.

MSO was administered intravenously, topically and intraventricularly. The stationarity of the recorded neuronal output was considered to be sufficient on account of the precautions taken during registration and the qualitative analysis of the course of the number of action potentials per 6 sec.

The overall mean firing frequency for all the neurons recorded in the sensorimotor cortex of the rabbit amounted to 5.2/sec. which is in good agreement with data from the literature. A relation with depth of the mean firing frequency was demonstrated in depth classes of 200 micron in width.

For 15 out of a sample of 23 neurons independency of successive intervals was assumed on account of the used statistical tests, indicating that analysis of the interval histogram on a base of independency of successive intervals is allowed while, moreover, an explanation can be found for the dependency, causing bimodal interval histograms in some neurons.

The quantitative analysis of the interval histograms of the group of normal neurons showed a relation with depth of the first moment and with the coefficient of variation. A high correlation was seen between the first and the second moment and between the β_1 and β_2 measures for skewness and curtosis, respectively.

The mathematical method of curve-fitting showed that when 80% of the total surface under the curve was used, 34% of the unimodal interval histograms fitted to a truncated gamma-distribution at a 0.5% level. This was considered to be very high when taking into account the high power of discrimination of the chi-square test used in this method. Moreover the arbitrarily chosen level of 80% is rather coarse when looking for the best point of truncation.

After intravenous injection of MSO a behavioral pattern develops which agrees more or less to data from the literature and ends in an epileptic state. In the rabbit, episodic running behavior always immediately precedes the convulsion, the time course is dose-related and covers plm. 60 hours till the onset of convulsive activity. When MSO is injected intracisternally, though, the whole behavioral pattern is finished within 2 hours. The influence on the EEG of the administration of MSO is very evident giving profound changes before the onset of the convulsions. The effect on the

unit activity is much less clear, at least before the onset of the convulsions. Following administration of high doses of MSO intravenously, the EEG consists after 3-3.5 hrs exclusively of patterns of convulsive activity separated by periods of electrical silence. This can also be seen in the firing activity of neurons recorded during convulsive activity. Topical application demonstrated no effect whatsoever while, after intraventricular administration, a very quick onset of changes in the EEG was observed, lasting up to one hour. With the qualitative methods used, no differences in unit activity between the control and the MSO-neurons could be demonstrated.

The results of this investigation have been published in a doctoral thesis.

Dr. R. Singh (Research-fellow IBRO/UNESCO):

In order to acquire a general familiarity, proficiency and practical working knowledge of various neuro-electrophysiological techniques and instrumentation, some basic experiments on recording and stimulation techniques were done first. Both macro- and microelectrode techniques were studied. Thereafter, techniques of electrode implantations in the brain of rats, and EEG recording were mastered. Knowledge of computer analysis of bioelectric events was acquired and techniques of studying drug effects on the electrical activity of brain (EEG) were studied. Consideration was also given to cerebrovascular functions and telemetric recording of EEG activity.

With a view to formulate a specific research project, the investigations were directed towards studying the effects of some drugs on the electrical activity of the brain (EEG). Since a correlation between the brain electrical activity and its circulation-metabolism resulting from the reduced blood flow is often found in pathological and similar situations, it was considered useful to study some of the patent vasodilator drugs which are thought to improve cerebral circulation in cerebral disorders resulting from the reduced cerebral blood flow.

Nicotinic acid, cyclospasmol, ronicol, etc., were used in this work. The effects of these drugs were investigated on the EEG of rats chronically implanted with cortical and subcortical electrodes. At first the effects of these drugs on normal brain EEG in unrestrained, unanesthetized and anesthetized rats were studied. Observations were made of the animals kept in the recording chamber which was sound proof, and dimly lighted with one way glass. Motor behavioural activity of the animal under experiment was always taken into account.

Since direct measurements of cerebral blood flow in the brain of rabbits by other investigators in this laboratory had indicated that nicotinic acid decreased the cerebral blood flow by about 25% thus probably resulting in the loss of autoregulation, it was considered of importance to note first the effect of nicotinic acid on the EEG and then to see the influence of vasodia-

lator drugs in nicotinic acid treated animals. To some extent this provides a close relationship to the clinical situations involving decreased cerebral blood flow and the expected or suggested improvement of the latter by treatment with the drugs.

Nicotinic acid was found to induce a sleep-like state in the rats. This effect was preceded by a marked reduction in the motor activities of the animal, *i.e.*, disappearance of most of the usual automatic and voluntary movements. This effect lasted for about two hours. The EEG tended to be like that in normal sleep and drowsiness. Adequate control studies were always made. Autocorrelation and subsequent Fourier analysis of the EEG of the normal and drug-treated states did not reveal any further significant information about the concerned EEG patterns except that the EEG of nicotinic acid-treated animals appeared to be more synchronized and rhythmic for most of the time than was true in the untreated animals.

This nicotinic acid effect was relieved by cyclospasmol, *i.e.*, when cyclospasmol was administered to nicotinic acid-treated animals the above mentioned nicotinic acid effects did not appear. Ronicol was not as effective as cyclospasmol.

Direct monitoring of cerebral blood flow in order to observe correlation between the EEG and the CBF (cerebral blood flow), and experiments to study the effects of other drugs of similar properties should be performed. It would be necessary to extend these investigations in order to arrive at definite conclusions.

Drs. L. W. Zoeter :

At the moment a central position is taken up by the concept "muscle tonus", by which is meant the resistance of the muscle when being stretched as it is measured by the physician by means of passive movements in a joint. In a state of rest a certain amount of tonus or tension of the muscle appears also to be present during palpation. It is generally assumed that in both situations mentioned this tension depends on a neural and reflexogenic influence. An active contraction of small units of muscle fibers causes and sustains the tension in the muscle. This view is especially based on animal physiological data.

All the same it appears from an EMG examination that a muscle at rest in normal circumstances does not produce any electrical discharges, for which reason alone an active contraction to sustain the rest tonus is hardly probable.

In the case of a muscle which is stretched, the phasic and tonic stretch reflexes are supposed to play an important part. In order to test this an instrument is developed at this Institute which, on the one hand, will make it possible to cause a movement of an extremity which is determined as to speed and distance while, on the other hand, a continuous registration can be obtained of the forces developed in this process. Especially the resistance which is produced by the extremity in question, is expressed in

absolute terms. These data will be compared with the muscular contraction which is either or not present, measured by means of the integrated electrical output.

2. Section of Comparative and Developmental Neurophysiology

Dr. M. A. Corner :

The following studies were performed:

- a. The extent of spontaneous "struggle" movements in the chick as function of post-hatch age, and possible correlation with "paradoxical" phases in the cerebral electrical activity during sleep (paper in preparation with W. Bakhuis).
- b. Microscopic study in Golgi-Cox preparations of the dendritic and axonic development of cerebral neurons in the chick embryo.
- c. Measurement of the "EEG survival time" in the cerebral hemisphere after decapitation at different stages of pre- and post-hatch development in the chick (in connection with a biochemical study of cerebral metabolism; see report Dr. J. F. Jongkind).
- d. Study from filmed records of bioelectric activity in small neural systems developing *in vitro*: measurement, as a function of time in culture and origin of the tissue, of the thresholds for evoking stereotyped poly-synaptic activity and the interval distributions for *spontaneous* occurrence of (*similar*) spike/wave barrages (paper in preparation with Prof. S. M. Crain).
- e. Origin and mechanism of arousal systems using brain stem electrical stimulation in (sleeping) restrained vs. unrestraint chicks.

Drs. A. P. C. Bot :

The results obtained during the last two years of experimentation concerning cerebral seizures induced in the developing chick embryo have been extensively analysed this year. From EEG records, continuously taken from the animals for at least 6 hours after topical application of the drugs methionine sulfoximine (MSO), ouabain and pentylenetetrazol, measurements were made of several parameters of the patho-physiological phenomena. The latency times, duration of the seizures, intervals between seizures and the spike frequency during seizures were all subjected to statistical analysis. Twenty embryos were studied for each drug at each stage, beginning with ca. 1.5 weeks *in ovo* until hatching, enabling the critical periods during development for first generating seizures with each different drug to be studied. Studies of this type in the literature are always purely descriptive, without any attempt at quantitative treatment. In our investigation we tried to make an extensive statistical approach of the different parameters to compare the different drugs. It was decided not to compare the stages by means of lumping all of the observations made of each given parameter, but to calculate separately for each embryo

the mean and variance of each seizure parameter. The obtained means are handled as observations and used for significance analysis, with the Krushal and Wallis and with the Wilcoxon tests, because it was found that the parameters were distribution-free. The advantage of so treating the results is the attainment of a good impression of the changes in given physiological parameters during development. At the same time differences will be revealed between the several drugs in their effect on the developing brain. The results of this study are to be published in a thesis.

Drs. H. A. A. de Jong :

The study of statocyst-induced neural activity in snails (*Helix pomatia*) has been continued, using quantitative methods. Convincing behavioural evidence for gravity perception in these animals has been found:

1. compensatory head-orientation;
2. compensatory movements of the eye-bearing tentacles following changes in spatial orientation of the body;
3. genuine geotactic movements, *i.e.*, locomotions clearly related to the direction of gravity.

Statocyst-extirpation experiments point to a dominant role played by these organs in the geotactic behaviour.

This stimulus-response system has next been used for analysis of the transmission of signals in the central nervous system. In the recordings of the neuronal activity of the static nerve (*i.e.*, the *input* to the CNS) and of the cerebral-pedal connective (*i.e.*, *one* of the outputs of the cerebral ganglion) there arises a response to stimulation of the preparation by movement.

- a) Artifact-free registration of electrical activity from the nerves over many hours is possible by means of a specially developed electrode technique.
- b) A precise description of the activity patterns found in the static nerve is necessary for revealing these response phenomena.
- c) Action potentials of consistently different height arise from the axons (only 8-12, of different sizes). These are directly connected with the sense cells in the statocyst.
- d) An adapted multichannel analyser system shows an "amplitude spectrum" composed of the different single units that occur in the recordings from the static nerve. These spectra are specific for given spatial orientations preparation.
- e) This analysis technique gives the interrelations between the activity of the different sense cells, the activity evoked in the CNS by these units, and the spatial orientation and/or distinct movements of the preparation.

Further registration and analysis is projected for this investigation.

Ir. J. Smith :

Various functional aspects of the central nervous system have been approached with statistical mathematical techniques as a part of multi-disciplinary studies. The central theme of these investigations is the CNS as an information processing system. A study of activity on the neuronal level is initiated, in collaboration with H. van Wilgenburg, in the CNS of the snail, *Helix pomatia*. The neuronal activity is considered as a consequence of intrinsic and synaptic noise phenomena.

A second investigation is centered on the hypothalamic-hypophyseal relations. Simulation is performed with the IBM 1130 Continuous System Modelling Program, in collaboration with Dr. J. P. Schadé.

We are preparing micro-electrode techniques in the hypothalamic nuclei to reveal some of the intricate relations between neuronal activity and chemical stimulation with several hormones. A multi-barrel electrode set up has been developed for this purpose.

Finally, some software has been developed for the general support of the physiology department. This regards the analysis of continuous signals like the electroencephalogram and the mathematical treatment of isotope clearance curves for the calculation of cerebral blood flow, in collaboration with Dr. J. C. de Valois en Drs. J. P. C. Peperkamp.

Drs. H. van Wilgenburg :

A study was made about spike initiation, propagation and transmission in the neurons of the CNS of the snail *Helix pomatia*, these neurons being specially suitable for this aim. A preparation consisting of the ring of ganglia and the nerves connected with the mantle, is used for intracellular recordings with glass capillary micro-electrodes and for extra-cellular recordings with silver wire electrodes.

The occurrence of different "neurotransmitters" is being studied with biochemical and histochemical methods, in collaboration with Dr. J. J. Meisch. Acetylcholine, serotonin and dopamine, as possible transmitters, were perfused through the specially designed chamber (Van Embden). The influence of these substances on the spike activity is compared with the postsynaptic potentials.

Local application of these and other substances is now also being studied with multibarrel-electrode techniques (Van Leeuwen).

Statistical analysis of spike trains with computational methods are performed in collaboration with Ir. J. Smith, in order to understand the role of membrane noise and synaptic noise in neural information processing (publication in press). The neuronal precision of single units is also studied on receptor hairs on the cerci of the cockroach (J. Augustus).

The localization of the spike-initiating zone is described for a cell which exhibited an axonal reflex mechanism (1 and 2).

Electrophoretic injection of the cells with the fluorescent dye, procion

yellow, makes it possible to follow the cell processes. These, along with transient-averaging techniques (used to recognize the activity of a given cell in the nerves) give a good insight in the three dimensional structure of the neural system. The network properties are approached by careful analysis of input and output of the nervous system with cross correlation techniques and monitoring the overall states of activity of the cell assembly.

A multibarrel electrode set up is being developed for a multidisciplinary study of hypothalamic nuclei in the rabbit.

3. Section of Behavioral Physiology

Drs. H. van Dis and N. van der Poll :

Two distinct but closely related lines of research have been developed. The first centers on the influence of manipulation of the levels of circulating testosterone upon intracranial selfstimulation in the rat. The stimulated areas were the medial preoptic area which is reported to be of major importance in the regulation of the male sexual behaviour, and the lateral hypothalamic area, which is essential in the regulation of feeding behaviour. Preliminary analysis of the results has shown a differential effect of testosterone on the selfstimulation rate in both areas.

The second line was centered on the function of the hypothalamic-hypophysial-gonadal axis in the regulation of male sexual behaviour in rats. There is contradictory evidence about the effect of supramaintenance dosages of exogenous testosterone on the different parameters of male sexual behaviour. Therefore, this problem was reinvestigated. Furthermore, a project was started to study the influence of exogenous LH (luteinizing hormone) on male sexual behaviour in rats.

Drs. A. P. van der Meché and V. Kappers :

During the first half year of 1969 the central part of an automatic learning device for fishes has been completed. Experiments were started in order to trace the optimal conditions for the learning procedure.

Our experiments, and those of others known from the literature, show a strong correlation between spontaneous crossing of the shuttlebox barrier by the fish and "learned crossing", i.e., crossing of the barrier after application of a certain stimuli (light, sound) to the fish. If the number of spontaneous crossings during a fixed time interval increases, the amount of learned crossings = positive responses increases as well.

This correlation makes it difficult to tell what is happening actually. Is a high number of positive responses due to learning, or is it merely the result of a greater probability of crossings due to the high level of spontaneous responses?

At this moment, spontaneous behaviour in the shuttlebox of two species of fish, the goldfish *Carassius auratus* and the trout *Salmo gairdneri*, is studied. No stimulus is applied during the fishes' stay in the experimental

environment. A mathematical description of this behaviour is to be made, enabling to predict the probability of undisturbed spontaneous crossing during a conditioning experiment.

Drs. L. H. J. Meijs :

The literature dealing with effects of forebrain- and cerebellar lesions in fishes clarifies little or nothing about the role of these organs in behaviour. In general, in the experiments concerned, the influence of rather large lesions on classical and operant conditioning was studied.

This year a different approach to the same problem of cerebellar and forebrain function in fish was attempted. In principle, a fish could pay attention to thousands of stimuli but since the "channel capacity" of the nervous system is limited, only a selected part of the total information can be taken up. The cerebellar and forebrain cortex may have much to do with this central mechanism of selective attention.

For this purpose the behaviour of the three-spined stickleback in a T-maze was studied. On the choice point in this maze, the subjects can react to visual discriminanda (red or green colour), or to position (left or right). In tests with intact and impaired animals a significant tendency in responding to position was observed, whereas the animals remained indifferent to colour cues.

Some evidence is growing that by means of small lesions in the cerebellar or forebrain cortex in specific places, the balance can switch from responding to position before operation to responding to colour cues after operation. Much work has to be done before this way of studying cortical functions will become fruitful.

Drs. E. Pouwels :

The normal behaviour of the male three-spined stickleback during the parental phase of the reproductive cycle can be altered by cutting parts of the branchial nerves IX and X (Segaar, 1966 * and personal communication), *e.g.*, the fanning activity is much increased or diminished. Segaar assumed a sense organ in the pharynx region, taking up chemical stimuli released by the eggs. This was investigated by histological techniques. In the epithelium of the pharynx mucous cells, taste buds, so called spindle-shaped cells and "free" nerve endings were observed. These elements are dispersed over the whole oral cavity and pharynx. Acetylcholinesterase was demonstrated in the nerves and the spindle-shaped cells.

It is not yet possible to eliminate only one type of these structures without impairing the animal too much to test its behaviour. Thus, the histological findings and the behaviour of the male animal during the reproductive period could not be related directly.

*) Segaar, J.: Umschau in Wissenschaft und Technik 10, 333 (1966).

DIVISION OF NEUROCHEMISTRY

Dr. J. Vos :

Our work on acid mucopolysaccharides (AMPS) was continued. The subcellular distribution, total concentration and nature of the different acid mucopolysaccharides was established and confirmed by repeated experiments. The adult rabbit brain contains 0.025% AMPS on a wet weight basis and consists of chondroitin sulfate (65-70%), hyaluronic acid (20-25%) and small amount of possibly dermatan sulfate.

The distribution was 53% in the 100.000 g. supernatant, 16% in the nuclear fraction, 13% in the crude mitochondrial fraction and 11.6% in the microsomal fraction. Subfractionation of the crude mitochondrial fraction showed 1.8% in myeline, 6.6% in the synaptosomes, and 2% in purified mitochondria.

In the synaptic vesicles fraction we recovered 7.7%. This distribution indicates that the AMPS behave as a very soluble compound.

This part of the investigation concerning the quantitative distribution has been finished. A publication is in preparation. Investigations on changes in AMPS content in the developing brain of the rabbit were started in order to find out whether the AMPS behave as most compounds in the brain do: usually increasing in concentration from birth to maturity. To our surprise we found that at birth the brain contains 0.1% AMPS on a wet weight basis, while at 60 days this concentration is 0.025%. This means a 4-fold decrease. At 10, 20 and 30 days this concentration was 0.08%, 0.07%, 0.05% respectively.

The subcellular distribution during development is rather similar to that at the adult stage, the concentration in the microsomes only showing an elevation in the young animals to about 30% as compared to about 12% in the adult animal. Such a decrease in the concentration of a compound during development has, so far, never been observed and poses some very interesting questions as to the function of these molecules. We hope to obtain more information in 1970.

With regard to the function of AMPS in binding inorganic or organic cations or influencing their activities, we started a physico-chemical investigation of their properties. By means of two half-cells separated by a cation-exchange membrane it is possible to study the binding of Na^+ , K^+ , Mg^{++} , Ca^{++} , acetylcholine and noradrenaline by measuring the potential changes across the membrane. It is obvious that chondroitin sulfate can bind large amounts of acetylcholine and noradrenaline. In the next year we will investigate the influence of Na^+ , K^+ , Mg^{++} and Ca^{++} on the binding of transmitter substances to the AMPS.

Experiments in cooperation with Dr. J. Drukker from the Department of Anatomy and Embryology of the University of Amsterdam are now almost finished. In chick embryos, incubated 72 hrs, one eye was removed.

At the 10th, 14th and 20th day of incubation the embryos were sacrificed and the histological (Dr. J. Drukker), biochemical and enzyme histochemical effects of the operation studied in the telencephalon and in the optic lobes on the operated as well as on the non-operated side. The general protein content and the activity of acetyl-cholinesterase, lactate dehydrogenase, lactate dehydrogenase fumarase, uridine triphosphatase, thiamine pyrophosphatase and acid phosphatase were determined.

It was found that, in the telencephalon, the determinations showed normal values on both sides. Notwithstanding clear histological and enzyme histochemical differences in the optic lobe contralateral to the removed eye as compared with the homolateral optic lobe, no quantitative biochemical differences were present in both, the optic lobe at the operated and that at the non-operated side.

DIVISION OF NEUROPHARMACOLOGY

Dr. J. J. Meisch:

Previous investigations were continued along two main lines:

1. One research project consists of studying the influence of various drugs on the nervous system of invertebrates. Results were obtained with reserpine - like substances on the ganglia of the snail, *Helix pomatia*. Nevertheless it was found that the dopamine and serotonin determinations should be refined in order to use less animals. At present we are trying out a two column step procedure for chromatographic separation. An attempt is also made to increase the sensitivity of the spectrophotofluorometric determination of dopamine. When these rather difficult technical problems are solved, interesting drug studies can be achieved. This work will be carried out together with a group of physiologists at the Institute.
2. A second research project concerns false transmitters such as metaraminol and α -methyl-m-tyramine and their fate in the mammalian brain (rats and rabbits). The influence of various drugs such as anti-depressive drugs, reserpine, β -hydroxylation inhibitors will be studied. Again, at the present stage preliminary technical problems have to be solved such as appropriate fluorometric determination combined with column chromatography.

J. ARIËNS KAPPERS,
director

HUBRECHT LABORATORY

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PROGRESS REPORT 1969*

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I. EARLY AMPHIBIAN DEVELOPMENT

a. *Experimental morphology and cinematography*

1. The induction of mesodermal and endodermal structures from the animal, "ectodermal" part of the blastula by the vegetative, endodermal part (*Ambystoma mexicanum*, *Triturus alpestris*) (P. D. Nieuwkoop)

As stated in the previous report (sect.I.a.1) the mesoderm of the amphibian embryo arises from the animal, "ectodermal" half of the blastula as a result of an inductive action emanating from the vegetative,

endodermal half (see sect. VIII, ref. 7,8). This year xenoplastic recombination experiments were carried out involving animal, "ectodermal" and vegetative, endodermal material of the blastula stage. It could be shown conclusively that the vegetative endoderm does not contribute cellular material to the future mesoderm, but only acts as the inductor of the latter. On the other hand, the animal "ectoderm" furnishes the material for all the induced mesodermal structures, and moreover sometimes contributes considerably to the later endodermal structures, particularly the pharynx and the dorsal intestinal wall. As was already known, the "ectoderm" also forms the proctodaeum, the cells of which acquire a rather typical endodermal appearance.

2. The induction of the mesoderm in the anuran blastula (*Xenopus laevis*) (S. Sudarwati - doctoral thesis subject, Bandung Institute of Technology)

The *Xenopus* embryo is particularly suitable for quantitative analysis because only 24 hrs. of cultivation are required for identifiable cellular differentiation; consequently isolates and recombinates (Harrison stage 9-) hardly lose any cellular material during cultivation, and the absolute amounts of ecto-, meso-, and endoderm can be determined. The analysis showed unequivocally that also in anurans the mesoderm originates entirely from the "ectodermal" part of the blastula under the inductive influence of the endodermal part. The outer and the inner layer of the "ectoderm", when combined separately with the endoderm, show the same mesodermal competence, i.e., the same proportion of the "ectoderm" becomes mesodermized. The outer layer in addition becomes partially endodermized. On the other hand, the neural competence of the outer layer is considerably lower than that of the inner layer. It is possible that the permeability of the cells of the outer layer is markedly different from that of the cells of the inner layer, but this assumption still has to be tested experimentally.

3. The regional inductive capacity of the blastula endoderm (*Ambystoma mexicanum*) (E. C. Boterenbrood)

Preliminary experiments carried out in 1968 had shown that in the blastula of stage 8+ (Harrison) both the dorsal and the ventral endoderm are capable of inducing a wide range of mesodermal structures from the "ectoderm", but that notochord and muscles are induced more frequently by the dorsal endoderm. In later stages the dorsal endoderm loses its mesoderm-inducing capacity earlier than the ventral endoderm.

This year more experiments were carried out at stages 8+ - 10, in order to study the possible correlations between the character of the endodermal differentiations produced by the inducer and the induced mesodermal structures. In addition, the regional decline of inductive capacity in the endoderm was followed in more detail.

4. The induction of gastrulation (*Ambystoma mexicanum*) (M. H. M. Doucet-De Bruine – doctoral thesis subject, Univ. of Utrecht – with financial support of the Netherlands Organization for the Advancement of Pure Research)

As stated in the previous report (sect.I.a.1) indications have been found that the first phenomena of gastrulation in the vegetative half of the blastula are induced by the animal half. In vegetative halves isolated at stage 9-10 (Harrison) (late blastula) the cells in the prospective blastoporal region elongate at the same time as the prospective blastoporal cells in intact control blastulae. The formation of morphologically and histologically typical “flask” cells first occurs at the dorsal side of the isolate and then proceeds to the lateral and ventral sides. This phenomenon has now been found to depend on the stage of isolation: the younger the stage, the shorter the cells and the less ventral the extension of flask cell formation. Vegetative halves isolated at stage $7\frac{1}{2}$ (early blastula) are not yet capable of flask cell formation unless a narrow ring of the animal half is included in the isolate.

Experiments have been started to test the possibility of evoking flask cell formation in purely vegetative isolates of stage $7\frac{1}{2}$ by recombining them with prospective ecto- and mesodermal material of the same or other stages.

5. Cinematographic study of the cleavage pattern (*Ambystoma mexicanum*) (K. Hara)

A “double camera” time-lapse film apparatus was constructed, with which one egg can be filmed simultaneously from both the animal and the vegetative side (see ref. 17).

Analysis of six pairs of films taken by this method revealed the following features (at 20-22 °C). About 30 min. before the appearance of the first cleavage furrow a narrow circular band suggesting a zone of surface contraction originates at the animal pole. It moves centrifugally, passing the equator of the egg and reaching the vegetative pole in about 100 min., i.e. about 30 min. before the first cleavage furrow reaches the vegetative pole. A second similar wave starts at the animal pole about 10-15 min. prior to the initiation of first cleavage; it also extends centrifugally, but is observed in the animal hemisphere only. About 10-15 min. prior to the initiation of both the second and third cleavage similar waves start from the future initiation points of these cleavages; they are likewise confined to the animal hemisphere.

The cleavage pattern itself shows the following features: (1) During the first 12 or 13 cleavage cycles the cell divisions occur in synchronous waves, starting at the animal pole and proceeding towards the vegetative pole. From the 13th or 14th cleavage onwards the cell divisions become asynchronous. (2) The synchronous cleavage waves succeed each other at inter-

vals of about 80 min. (at 20-22 °C). The time elapsing from the start of the 13th cleavage until the onset of gastrulation is about 300 min. (3) The duration of the cycles is independent of egg size (range of egg diameters 1.9-2.3 mm.).

6. A more accurate staging method for the morula and blastula (*Ambystoma mexicanum*) (K. Hara and E. C. Boterenbrood)

Harrison's normal table for *Ambystoma punctatum* gives only three stages for the morula and blastula (st.7-9). Moreover, the criteria for these stages are rather vague, so that interpolation of extra stages entails considerable inaccuracy. In order to arrive at a more detailed staging based on quantitative criteria, several films of cleaving eggs taken by the "double camera" technique described in the preceding section were used. Drawings were made of the animal hemispheres just prior to the start of each new cleavage wave, and the number of cells intersected by the egg diameter was determined. From the 10th cleavage onwards counts were based on half the egg diameter (one quarter on either side of the animal pole), because cells situated peripherally are difficult to count. In this way the developmental period comprised by Harrison stages 7-9 was divided into eight stages characterized by a step-wise increase in the number of cells (each step coinciding with one cleavage wave).

b. *Electron microscopy*

1. Electron microscopic study of the first cleavage of the egg (*Ambystoma mexicanum*) (J. G. Bluemink)

At the animal pole of the first-cleavage zygote the future plane of furrowing is first indicated by a stripe of pigmentation, which some minutes later splits into two. The area between the stripes deepens and widens, and on both sides stress-marks appear in the egg surface. These smooth out again when a shallow groove develops. Electron micrographs revealed that the egg surface in the region of the single and the double stripe is greatly enlarged by surface protrusions. In the cortex previously non-oriented filaments become polarized and aggregate. Light vesicles which apparently arise at the expense of stacked membranes from the diastema form a cortical layer. The pigment granules show changes, while in the subcortical cytoplasm yolk granules undergo degradation.

These structural alterations are thought to represent the mobilization of cytoplasmic pools, giving rise to the appearance of multivesicular bodies, dense bodies, and cortical granules. As a result of constricting surface movements wrinkles and folds come into being. Cytoplasmic areas packed with osmiophilic stacked lamellae are found exclusively in that region of the primary furrow which is known to contribute directly to the formation of new surface. A functional relationship between the light vesicles and the layer of filaments is postulated, and can be related to the mechanism by which the karyokinetic event initiates furrow formation.

At present the same stages of first cleavage are being studied in the egg of *Xenopus laevis* for comparative purposes.

2. Electron microscopy of cortical wound healing in the egg (*Ambystoma mexicanum*) (L. Luckenbill)

Holtfreter (1943) found that upon mechanical disruption of the egg cortex the wound edges are first drawn wide apart, but that very soon the wound area is reduced by surface contraction. An electron microscopic study of this process showed that some cytoplasm is extruded through the wound. Fat droplets, pigment granules, and disintegrating yolk granules accumulate at the wound surface. In the transitory zone between the wound and the adjacent cell surface the exovate is engirdled by a filamentous "belt" lying just below the cell surface. Light vesicles are present in large numbers in this zone. It is thought that the filamentous belt is the site of the constricting surface movements which lead to wound closure. This interpretation is at variance with Holtfreter's conception that the structural basis for the elastic properties of the egg cortex is located in an extracellular, so-called "surface coat". (Investigation to be continued in the U.S.A.)

c. *Histo- and cytochemistry* (including autoradiography)

1. Early chemical differentiation in various regions of the blastula and early gastrula (*Ambystoma mexicanum*, *Xenopus laevis*) (G. A. Ubbels)

As stated in the previous report (sect. I.c.2), preliminary investigations had brought to light a cytochemical heterogeneity of the cell populations of the prospective ectoderm in the blastula, and of the ectoderm and mesoderm in the early gastrula with respect to (muco)polysaccharide content. The origin of this heterogeneity is now being studied in *Xenopus*. At the same time an attempt is being made to further characterize the substances involved.

The amount of stainable material varied considerably between different egg samples. Therefore the staining method was improved and standardized so as to be able to show the heterogeneity also in weakly-staining embryos.

2. Autoradiographic study of mesoderm induction in the blastula (*Ambystoma mexicanum*) (G. A. Ubbels and P. D. Nieuwkoop)

The nuclei of early blastulae (Harrison stage 7½) were labelled with ³H-thymidine by means of Chibon's injection method. The blastulae were allowed to develop till stage 9, and parts of them were then recombined with parts of unlabelled blastulae of the same stage. The preliminary results support Nieuwkoop's hypothesis concerning the origin of the mesoderm (see I.a.1 above).

3. Cytological study of the distribution of yolk platelets in the oocyte and the fertilized egg (*Xenopus laevis*) (R. W. Glade)

This study was carried out as a basis for further experimental work concerning the effect of locally applied heat on the distribution of cytoplasmic inclusions and on the cytochemistry of the egg. In the mature oocyte yolk platelets occur in a continuous range of sizes from 1 to 13μ (average diameter), with the majority having average diameters between 1 and 3μ . Along the animal-vegetative axis the oocyte is polarized with respect to platelet size rather than number: small platelets predominate in the animal hemisphere, while large platelets (with some admixture of smaller ones) are typical of the vegetative hemisphere. In the animal hemisphere a peripheral zone of densely packed small platelets is a conspicuous feature. Between the two main regions there is a transitional zone of medium-sized platelets with rather abrupt boundaries towards the animal and vegetative hemispheres. This transitional zone forms a shallow cup with the rim slightly elevated toward the animal pole.

In the fertilized egg the platelets in the animal hemisphere seem to form a dorso-ventral gradient with respect to size and distribution. Dorsally the platelets in the peripheral zone are about twice as large as ventrally, while frequently the peripheral zone itself is broader. Moreover, the rim of the transitional zone of medium-sized platelets is more elevated on the dorsal side.

These observations suggest that fertilization might initiate a cytoplasmic streaming movement resulting in a dorsal- and animalward displacement of the medium-sized yolk platelets. To test this hypothesis a suspension of polystyrene spheres of a similar size range as the yolk platelets was injected immediately upon fertilization into the egg through the vegetative pole, the needle tip reaching just beyond the zone of medium-sized platelets. The distribution of the spheres was studied later in sections. The preliminary results seem to support the hypothesis. (Investigation to be continued in the U.S.A.)

d. *Biochemistry*

1. Isolation and characterization of embryonic ribosomes (*Xenopus laevis*, *Ambystoma mexicanum*) (P. Th. M. van der Saag and H. Nederbragt)

The main difficulty in the isolation of ribosomes from amphibian embryos is the presence of large quantities of glycogen particles, which largely obscure the population of ribosomal particles. Several methods for removing glycogen specifically were tested. Enzymatic digestion proved not to be very suitable, because of the relatively high incubation temperatures required and the contamination of most glycogen-digesting enzyme preparations with proteolytic enzymes and RNase. A more promising method seems to be to use certain plant proteins that form specific

complexes with polysaccharides, which can then be removed by low-speed centrifugation.

Another abundant egg and embryo constituent that interferes with ribosomal isolation are the yolk proteins. In *Ambystoma* material a solution of high ionic strength (0.5 M KCl) is necessary to dissolve the yolk proteins prior to differential centrifugation. In *Xenopus* material, where yolk proteins are relatively less abundant, a solution of lower ionic strength is sufficient. Moreover, it was found that high ionic strength solutions prevent the isolation of "healthy" ribosomes.

Research on the possible changes in the properties of embryonic ribosomes during early development has been initiated.

2. Ultrastructure of ribosomal and glycogen particles in amphibian embryos (P. Th. M. van der Saag and J. G. Bluemink)

In electron micrographs of intact early embryonic cells it is very difficult to discriminate between ribosomal and glycogen particles. This problem is being approached by studying the ultrastructure of pellets of cell organelles obtained by differential centrifugation of homogenates.

3. Isolation and characterization of RNA from amphibian embryos (P. Th. M. van der Saag)

A method has been elaborated which is based on step-wise thermal phenol extraction of RNA. Here also glycogen and yolk proteins greatly interfere with the extraction.

The characterization of the various types of RNA is being continued by means of disk electrophoresis in polyacrylamide gels, a method that has great advantages over that of sucrose density gradient centrifugation used so far.

4. Elaboration of culture methods for amphibian embryonic cells that permit the uptake of radio-active precursors (P. Th. M. van der Saag)

Three methods were compared with regard to practicability, reproducibility of results, and viability of the cells: 1) monolayer culturing of cells dissociated after removal of the vitelline membrane; 2) dissociation and reaggregation of cells inside the intact vitelline membrane; and 3) dissociation inside the vitelline membrane, followed by removal of the latter and monolayer culturing.

One of the problems with all three methods is instability of pH. Classical culture media for amphibian embryonic cells have a very poor buffering capacity. New buffers and media have been tested, but a definitive solution has not yet been found.

One of the criteria for the suitability of the medium has been the level of protein synthesis as measured by the incorporation of radio-active precursors. A major drawback is the fact that protein synthesis is influenced by the rate of reaggregation (restoration of the permeability barrier),

which is of particular importance when the vitelline membrane is left intact. Other – e.g. biological – criteria are also very variable. A combination of several independent criteria may bring the solution.

5. Protein patterns during embryonic development (*Ambystoma mexicanum*) (P. Th. M. van der Saag and J. J. Zeegers)

Analysis by disk electrophoresis in polyacrylamide gels has brought to light that the protein patterns of whole embryos of various stages (from fertilized egg till tail bud stage) show both qualitative and quantitative differences. Further study of protein synthesis at various stages by means of incorporation of radio-active precursors combined with disk electrophoresis depends on the development of a suitable culture method (see 4 above).

6. Separation of various cell types of the amphibian embryo (I. Slabý)

As stated in the previous report (sect. I.d.1c) a method was developed to separate early embryonic cells according to size and density by centrifugation in a Ficoll density gradient. The cells recovered from the gradient show protein synthetic capacity. Their differentiation upon reaggregation is being studied with histological techniques.

This technique, combined with the methods described in 4 and 5 above, seems promising for the study of spatial patterns of macromolecular synthesis in the embryo.

e. *Biophysics*

This year the new research unit of biophysics, headed by S. W. de Laat, has come into operation. The installation of electrophysiological equipment was continued, while at the same time the actual research started.

A beginning was made with measurements of electrical parameters of the blastomeres of early cleavage stages of *Ambystoma mexicanum* and *Xenopus laevis*. The membrane potential and the membrane resistance and capacitance are being measured.

At the same time preliminary experiments were made on the sodium and potassium metabolism of these early cleavage stages.

II. AMPHIBIAN DEVELOPMENT (general)

1. Pattern formation in the presumptive prosencephalon at the open neural plate stage (*Ambystoma mexicanum*) (C. F. A. Rosa and E. C. Boterenbrood)

The study mentioned in the previous report (sect. II.5) has now been extended to the lateral portions of the presumptive prosencephalic region. Small fragments from 20 different areas encompassing the entire presumptive prosencephalon were transplanted to a "neutral" region of host embryos to determine their differentiation tendencies. The quantitative analysis of the differentiated transplants is in progress.

2. Production and fate of new cells in the neural retina during metamorphosis (*Xenopus laevis*) (J. G. Hollyfield)

This work was an extension of work on *Rana pipiens* described in the previous report (sect.II.8). In *Xenopus* larvae the inner nuclear layer of the retina increases in thickness from two cells at stage 58 (Nieuwkoop & Faber) to three to four cells by stage 66 (end of metamorphosis). During this period the retina also increases in area concomitant with the increase in diameter of the eye. Colchicine treatment was used to locate the source of the new cells. Arrested metaphase figures were found at the margin of the retina and in the fundus.

Labelling of nuclei with ^3H -thymidine was used to determine the fate of the new cells by autoradiography. The cells produced at the margin are added to all three cell layers and account for the increase in area of the retina. The cells produced in the fundus accumulate in the inner nuclear layer and are responsible for the increase in thickness of this layer.

III. AVIAN DEVELOPMENT

1. The origin of the regional pattern of neural differentiation (*Gallus domesticus*) (K. Hara)

The experiments described in the previous report (sect. III.3), in which parts of the neuro-ectoderm were isolated, recombined with mesoderm from various levels, and cultured *in vivo* as "open sandwiches", were extended in number so as to allow a more detailed statistical analysis. The analysis is in progress.

2. Specificity of cell contacts during neural tube development (*Gallus domesticus*) (R. Adler)

Reaggregation experiments were performed with dissociated neural tube cells from 3-6 day old chick embryos. The procedures followed, as well as the first results obtained, were already described in the previous report (sect. VII.3). A comprehensive description of this and last year's findings follows.

Stage-dependent changes in the reaggregation pattern of the cells were found. While 4 day old optic lobe reaggregates develop a great number of epithelial rosettes after 24 hrs. in culture, rosettes do not appear in reaggregates of similar cells taken from 5 or 6 day old embryos. In cultures from these older embryos the most prominent morphological feature is the formation of a fibrillar layer at the periphery of the reaggregates. Reaggregates of 3 day old optic lobe (Hamburger & Hamilton stages 18-20) only exceptionally show rosettes, and never when optic lobe from stage 18 embryos is used. However, when cell suspensions of stage 18 optic lobe and 6 day old optic lobe are mixed, the resulting reaggregates show many rosettes.

The formation of rosettes in cultures of 4 day old optic lobe cells seems to be accomplished by invagination of "placode-like" structures present

at the periphery of the reaggregates during the first hours of culture. The cells in the rosettes resemble those in the early embryonic neural tube in their structure (both at the light and electron microscopic level), as well as in their mitotic behaviour. A more detailed study of the mechanisms of rosette formation is in progress. Experiments were performed in which ^3H -thymidine-labelled and unlabelled 3-6 day old optic lobe cell suspensions were mixed, and the results are being analysed by means of autoradiography and electron microscopy.

Labelling experiments using 6 day old optic lobe show that the DNA-synthesizing ("matrix") cells and the non-DNA-synthesizing ("differentiating") cells sort out, the latter cell type being found in higher numbers at the periphery of the reaggregates. Both the formation of a peripheral fibrillar layer and the sorting-out of the non-DNA-synthesizing cells towards the periphery in these reaggregates resemble processes occurring during normal neural tube development.

After 24 hrs. culture unlabelled 4, 5 or 6 day old telencephalon cell suspensions form reaggregates whose structure is similar to that of equivalent optic lobe reaggregates.

When 4-6 day old optic lobe reaggregates obtained after 24 hrs. culture are grown as chorioallantoic grafts for 8 days, many neuroblast-like cells are observed, but no indications are found of the formation of the concentric layers typical of the optic lobe. (Investigation to be continued in the Argentine).

3. Localization within the embryo of embryonic cells disseminated by the vascular route (*Gallus domesticus*) (J. G. Hollyfield and R. Adler)

Cell suspensions of either embryonic neural retina, optic lobe or liver, labelled with ^3H -thymidine, were injected into the extra-embryonic circulation of 7-day embryos. After 24 hrs. eight organs per host embryo were recovered and prepared as autoradiographs. The order of the host organs with respect to the number of labelled cells found per unit volume, independent of the cell type injected is: liver > kidney > (heart, stomach, lung, wing) > optic lobe > neural retina. The data indicate that the distribution of the labelled cells after 24 hrs. is neither random within the embryo, nor specific with respect to the type of cell injected.

In separate experiments, polystyrene spheres with sizes comparable to the above donor cell dimensions were injected into the circulation. After 24 hrs. six organs per host embryo were recovered. Significantly more spheres per unit volume were found in the liver and kidney than in the heart, stomach, lung or wing (see ref. 18).

4. Proportionate growth and the effect of thyroid hormone on embryonic limb bones in vitro (*Gallus domesticus*) (K. A. Lawson and co-workers)

The proportionate growth of the embryonic limbs is reflected in differences in overall growth rate of the individual cartilaginous limb-bone

rudiments, both *in vivo* and *in vitro*. As reported previously, differences in the growth response of the rudiments to triiodothyronine (T_3) *in vitro* are associated with the specific growth rates of the bones *in vivo*: the growth in length of fast growing bones, such as the metatarsus, is retarded by T_3 after several days in culture, while that of potentially slowly growing bones, such as the radius, is continuously stimulated. The metabolic differences between the $7\frac{1}{2}$ day old metatarsus and radius in the absence and presence of 10^{-7} M T_3 during 4 days culture are being investigated.

4a. T_3 and DNA synthesis (C. E. Hulstaert)

As reported last year (sect. VII.1.a), the DNA synthesis of T_3 treated rudiments is initially stimulated and then retarded; the metatarsus is both stimulated and retarded earlier than the radius. To see (1) whether these effects are due to changes in the number of cells synthesizing DNA, and (2) whether the response is localized, the total number of labelled cells was counted in autoradiographs of bones labelled for 1 hr. with 3H -thymidine after 12 or 96 hrs. treatment with T_3 .

In control bones the thin layer of connective tissue surrounding the rudiments accounted for about 50% of the labelled cells, the periosteum for 3%, the hypertrophic cartilage for 0.3%; the remaining labelled cells were distributed through the cartilaginous epiphyses and flattened cell zones.

After 12 hrs. treatment with T_3 the total number of labelled cells in the metatarsus was increased by 20%; after 96 hrs. it was reduced to 50% compared with the controls. The effects on the radius were not statistically significant. These results indicate that the effect of T_3 on the rate of DNA synthesis per bone is due, at least in part, to an initial increase in the number of cells synthesizing DNA, followed by a reduction in the number of dividing cells. Whether the initial increase is due to an increase in the population of dividing cells or to a reduction in the length of G, is not known.

Treatment with T_3 for 96 hrs. reduced the total number of cells in the flattened cell zones of the metatarsus to 30% of the control value; in the metatarsal epiphyses and the proximal epiphysis and flattened cell zone of the radius cell number was reduced to about 70%. There was no significant reduction in the length of the flattened cell zone after 96 hrs., suggesting that a reduction in the population of dividing cells occurs before the premature hypertrophy described by Fell and Mellanby (1956).

In contrast to the cartilage, the total number of labelled cells of the periosteum of the 96 hrs., T_3 -treated metatarsus was not reduced, and that of the radius was increased by 50%.

4b. T_3 and chondroitin sulphate synthesis (K. A. Lawson)

As mentioned in last year's report (sect. VII.1.c) the rate of incorporation of $^{35}SO_4$ per μg DNA is faster in the cultured metatarsus than in the radius, but the increased incorporation per bone in the presence of T_3 is relatively

greater in the radius. To test the possibility that turnover of chondroitin sulphate is relatively faster in the T_3 -treated metatarsus than in the radius, rudiments were labelled for 6 hrs. with $Na_2^{35}SO_4$, "chased" with carrier, and then cultured on unlabelled medium for a further 1, 2 or 3 days, after which the loss of incorporated activity was measured. About 7% of the incorporated activity was lost per day; there was no difference in relative loss between metatarsus and radius, and between T_3 -treated rudiments and controls. 3H -leucine incorporated into the TCA-insoluble fraction of the same rudiments disappeared at a rate of about 21% per day, irrespective of which rudiment was used and whether T_3 was present or not. It can be concluded that turnover of matrix does not differ in the two bones and is unaffected by T_3 . These results support the suggestion that differences between rudiments in their rate of synthesis of intercellular material are important in determining their response to T_3 .

In order to test whether the loss of incorporated ^{35}S was due to enzymatic action or leakage from the extracellular matrix, rudiments were double labelled with $^{35}SO_4$ and 3H -leucine, further incubated on unlabelled medium at 38 °C or 2 °C, the environmental pH being controlled with an air - CO_2 mixture, and the loss of radioactivity measured. The relative loss of ^{35}S and 3H (acid-insoluble) at 2 °C was only slightly less than that of ^{35}S at 38°, whereas the relative loss of 3H (acid-insoluble) at 38 °C was four times as great. It is suggested that the mucopolysaccharide-protein complex leaks from the matrix into the medium and that there is negligible enzymatic breakdown of the matrix under the culture conditions used.

IV. AMPHIBIAN LIMB REGENERATION

1. A re-investigation of distalization in transplanted limb blastemas (*Ambystoma mexicanum*) (J. Faber)

On the basis of the ultimate localization of carbon marks introduced into the tips of early limb blastemas prior to transplantation, Faber (1960) concluded that mesenchyme which normally never takes part in the formation of the digits may do so after transplantation of the blastema (distalization). Because this conclusion was based on a small number of cases, the experiments were extended; the orbit was used as the transplantation site, because this is known to support much better development of transplanted blastemas than the back (de Both, see previous report, sect. IV.2). Although the results are on the whole inconclusive, some cases suggest that limited distalization may occur.

2. Influence of innervation on the manifestation of the digits in transplanted blastemas (*Ambystoma mexicanum*) (J. Faber)

A re-examination of the material obtained by de Both in 1965-1968 (see previous report, sect. IV.2) showed that the experimental series fall

into two groups, those only containing transplants with three digits or less, and those containing 4-digit transplants as well. The second group consists of those series in which either blastemas transplanted to the back had been provided with a potent source of extra innervation, or blastemas had been transplanted singly to the orbit. In both cases silver staining of sections of the transplants showed that they were well innervated (in the second case presumably by regenerating eye muscle nerves). Transplants in the first group of series are very sparsely innervated. It may be concluded that the extent of innervation (of the epidermis?) has a specific effect on the number of digits formed by a transplanted blastema.

V. DEVELOPMENTAL GENETICS IN INSECTS

1. Genetics, morphology, and development of homoeotic mutants (*Drosophila melanogaster*) (W. J. Ouweneel – doctoral thesis subject, University of Utrecht – with financial support of the Netherlands Organization for the Advancement of Pure Research)

The experiments with the *loboid-ophthalmoptera* strain (see previous report, sect. V.1) were continued. This strain is characterized by homoeotic outgrowths from the eye area, consisting of wing tissue. A study of the differentiation capacities of fragments of normal and homoeotic optic imaginal discs by means of transplantation to full-grown wild-type host larvae ¹⁾ showed that the disc contains a mosaic of organ districts, each forming a specific part of the adult head. In the mutant strain the homoeotic tissue is formed by the presumptive eye area. That this differentiation is autonomous could be shown by *in vivo* culture of eye discs from newly hatched larvae in wild-type adult flies (see ref. 9, 10, 22, 23).

A beginning was made with transplantation experiments to test (1) the effect of colchicine treatment on the homoeotic phenomenon, and (2) the transdetermination capacities of imaginal discs from homoeotic mutant larvae (with the help of W. Lammers).

Some new homoeotic mutants were discovered. In a *Sevelen* wild stock flies appeared showing an *aristapedia* effect, probably due to a recessive allele of *Antennapedia*. The new mutation strongly enhances the effects of *Antp^B* and *Nasobemia*. In a strain containing the new mutation and *Antp^B* flies were found showing a strong *tetraptera* and some *bithorax* effect. Experiments were started to analyse the genetics, morphology, and developmental physiology of the new strains.

VI. DEVELOPMENT OF CELLULAR SLIME MOLDS

1. Chemotaxis in the *Dictyosteliaceae* (*Acrasieae*) (Th. M. Konijn)

As mentioned in the previous report (sect. VI.1) myxamoebae of *Dictyostelium discoideum* are chemotactically attracted by other myxamoe-

¹⁾ based on experiments performed by K. J. van den Berg in 1966

bae and by bacteria (see ref. 4). The sensitivity to attractants is highest when the myxamoebae are close to the aggregation stage (see ref. 2,4). The attractant was identified as 3', 5'-cyclic AMP (see ref. 5,6). The genus *Polysphondylium* and some species of *Dictyostelium* do not respond to this compound. At present the correlations are being studied between sensitivity to cyclic AMP and (1) aggregation size, (2) the occurrence of pulsations during aggregation, and (3) the absence of "founder cells".

Myxamoebae of *D. discoideum* are also attracted by certain analogues of cyclic AMP, but less strongly. Many analogues were tested (see ref. 5), and the results suggest that the 3', 5' ring is essential for chemotactic activity. The species which are insensitive to cyclic AMP also fail to respond to its analogues.

2. Analysis of specific and generic differences in sensitivity to cyclic AMP (*Acrasieae*) (Th. M. Konijn and M. Th. van Oldenborgh-van Grinsven)

Dictyostelium minutum and *Polysphondylium pallidum* are insensitive to cyclic AMP, but nevertheless respond to bacteria and their extracts. The attractants seem to differ from cyclic AMP in molecular size.

3. Chemotaxis in the true slime molds (*Myxomycetae*) (Th. M. Konijn and N. S. Nikitin)

Myxamoebae of the true slime molds are also chemotactically attracted by bacteria, but the analysis was complicated by the fact that bacterial extracts became less active at increasing purity.

4. Microbiological assay for the quantitative determination of 3', 5' - cyclic AMP in higher organisms (Th. M. Konijn)

It is known that cyclic AMP may play an important role in higher organisms as a "second messenger" in hormonal actions. Myxamoebae are being used in an assay for the determination of concentrations of cyclic AMP in tissue extracts. Amounts as low as 10^{-12} g can be measured, while the assay is sensitive enough to demonstrate twofold differences in concentration (see ref. 19). The following two projects were completed with the help of this assay:

- 4a. The role of cyclic AMP and catecholamines in melanophore pigment migration (*Xenopus laevis*) (with F. C. G. van de Veerdonk, Zoological Laboratory, University of Utrecht)

The mechanism of the melanophore dispersion reaction was studied. Darkened skin contained about twice as much cyclic AMP as light skin. It is postulated that pigment aggregation is dependent on phosphodiesterase, an enzyme that inactivates cyclic AMP (see ref. 24).

- 4b. The role of cyclic AMP in the action of parathyroid hormone on embryonic mouse calvaria (with Mrs. M. P. M. Herrmann-Erlee, Laboratory for Cell biology and Histology, Medical Faculty, University of Leiden)

Embryonic mouse bone tissue was exposed to parathyroid extract (one I.U. per ml) for various periods of time. Five to ten sec. incubation was sufficient to lead to a significant increase in the amount of cyclic AMP in the bone. The concentration of cyclic AMP was related to time of incubation and hormone concentration.

5. The incorporation of polysaccharide precursors into the cell surface of vegetative amoebae (*Dictyostelium discoideum*) (M. B. Burnside)

This project was an attempt to follow autoradiographically the incorporation of saccharide precursors into the cell surface. Vegetative amoebae of *Dictyostelium discoideum* provide a useful system for such a study because they do not secrete label into the medium during the exponential growth phase, which eliminates the complicating factor of export-polysaccharide synthesis. Amoebae were grown in suspension culture on dead bacteria and labelled during exponential growth with tritiated glucosamine, fucose, and galactose in pulse-chase experiments. Biochemical studies demonstrated that incorporation was into polysaccharide, with no significant conversion of the precursor to other monosaccharide. Light and EM autoradiographic studies on samples taken in parallel with those for biochemical analysis are in progress. It is intended to describe the process of surface saccharide incorporation during the steady state of exponential growth as a first step in detailing changes which occur during the developmental sequence of the slime mold. These changes, which can be very precisely controlled in shaker and roller cultures, involve distinct and well-characterized changes in adhesive properties of the cell surface. (Investigation to be continued in the U.S.A.)

VII. MISCELLANEOUS

1. Dr. W. P. Luckett studied material of the Hubrecht and Hill collections forming part of the Central Embryological Collection. The main object of his study was the comparative development of the placenta, fetal membranes, and extra-embryonic mesoderm in primates. In addition he examined the development of the placenta in marsupials in view of the relationships between metatherian and eutherian mammals. Finally, he studied material of several insectivore families in order to relate their placentation to that of the primates.

2. Dr. M. Klíma studied the development of the sternum in material of the monotremes and marsupials present in the Hill collection. His attention was focussed particularly on the contribution of the coracoid,

a bone which is fully developed in adult monotremes, while it is only present for a short time during development in marsupials and is completely absent in placental mammals.

3. Dr. K. I. Sirakami stayed at the Laboratory for a short period, mainly to study the Central Embryological Library.

P. D. NIEUWKOOP
J. FABER

VIII. PAPERS PUBLISHED AND ACCEPTED FOR PUBLICATION IN 1969

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15. BOTH, N. DE, Transplantation immunity in the axolotl (*Ambystoma mexicanum*) studied by blastemal grafts. J. exp. Zool.
16. HARA, K., "Dark-field" illumination for microsurgical operations on chick blastoderms *in vitro*. Mikroskopie.
17. ———, "Double camera" time-lapse micro-cinematography. Simultaneous filming of both poles of the amphibian egg. Mikroskopie.
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CENTRAALBUREAU VOOR SCHIMMELCULTURES
BAARN AND DELFT
PROGRESS REPORT 1969

In 1969 too the scientific research was mainly in the field of taxonomy. Only one of the major research projects could be completed during the course of the year. Parts of the other projects were finished and were laid down in a number of manuscripts. Sixteen publications appeared during 1969. Besides these there were twelve publications in press at the end of the year; three of them will appear as books.

Mrs. Dr. M. B. Schol-Schwarz, shortly before her death on 27th July 1969, completed her manuscript "Revision of the genus *Phialophora*", which with the help of Dr. K. W. Gams was made ready for publication. The species in *Phialophora* (synonymous genus *Margarinomyces*), were revised on the basis of their microscopical and cultural characteristics and reduced to twelve species or groups of closely related species. A connexion was established between some of the species of these fungi imperfecti and ascomycetes belonging to the genera *Pyrenopeziza*, *Mollisia*, *Ascocyne*, *Coniochaeta* and *Gaeumannomyces*.

Dr. G. A. de Vries initiated an investigation into *Cladosporium* and *Cladosporium*-like fungi. Seven strains of *Cladosporium werneckii* were tested for ability to form acid in phenol-red broth base + 4% of one of the sugars glucose, galactose, saccharose, maltose, lactose and raffinose (method of Wynne and Gott). It was found that some strains on galactose and/or rhamnose, after a good production of acid, formed so much basic substance (probably NH_3) that the pH changed to strongly alkaline.

New experiments with the fungus received as *Aphanoascus cinnabarinus* at various temperatures and on various media have still not led to a satisfactory formation of cleistothecia. It was found that the generally orange colonies often become bluish green on malt agar. A culture of this fungus was given to Dr. van Eyk for further study of this green pigment.

The pathogenicity tests on mice carried out by Dr. A. H. Klokke in Utrecht with three strains representative of a new species of *Trichophyton* did not result in the formation of lesions. Soil sampling along the ring dyke of Southern and Eastern Flevoland was continued with 72 samples. Still no keratinolytic representatives of the order Eurotiales have been found. One of the possible reasons for this phenomenon is undoubtedly the very high moisture content of the soil that despite the dryness of this

year was much higher than last year. Of the fungi isolated the most frequent were *Fusarium* (21 ×), *Alternaria* (12 ×), *Aspergillus fumigatus* (14 ×), *Streptomyces* (17 ×) and *Pythium*.

Miss Drs. A. C. Stolk with the cooperation of Dr. H. C. Evans (Keele, England) and Dr. T. Nilsson (Stockholm) investigated a new, brown, thermo-tolerant *Penicillium* species. Because of illness the work on a new classification of *Penicillium*, based mainly on microscopic characteristics, scarcely progressed.

Miss Drs. M. A. A. Schipper studied the possibility of dividing the genus *Mucor* into natural, phylogenetical groups of species. The results of matings showed that the divisions given by Zycha (1935 and 1970) are unnatural and unworkable. For instance, according to this author, *Mucor sphaerosporus* and *M. racemosus* would belong to two different sections because of the differing forms of the sporangiospores. In fact these two species closely resemble each other in all other characteristics and crossing between typical representatives yielded numerous zygospores. In addition, strains were found that formed round as well as oval spores and these intermediates alone make the separation of the two species unreal. On morphological grounds the species *M. chibinensis*, *M. plumbeus* and *M. fuscus* were placed in the same group as *M. racemosus*.

A second example of the close relationship between species with round and oval spores was found between *M. janssenii* and *M. griseo-cyanus*. Morphologically they can be distinguished from each other only with difficulty, notwithstanding the difference in form of the sporangiospores. That these species are closely related is also shown by the production of zygotes, on crossing.

The material present in the CBS collection was expanded with strains sent for identification or received from connexions and with our own isolates. Thanks to this material zygospores were obtained in *M. saturninus*, in a species related to *M. hiemalis* and provisionally identified as *M. lausannensis*, and in an unidentified species.

Mrs. Drs. E. J. Hermanides-Nijhof studied strains of *Fusarium javanicum* (*Nectria haematococca*). A newly acquired strain proved to be heterothallic. A series of single-ascospore cultures from one perithecium could be divided into two groups that did not form perithecia within themselves but did so with one another. The same results were obtained on repeating the crossings. Crossings were carried out between serial cultures from different perithecia to check whether there are indeed more than two groups. No results have been obtained yet.

The comparison of strains belonging to the group *Martiella* was continued. No correlation was found between the development and sporulation at different temperatures and the morphological characteristics of the strains examined. In addition, subcultures were made starting from small conidia

with few septa selected from a strain of a large-spored species (*Fusarium javanicum*). These again formed the same large spores as the original culture and did not change to cultures with only small spores.

Mrs. Drs. A. J. van der Plaats-Niterink pursued her study of the *Pythium* species occurring in the Netherlands. Continuing the mating experiments revealed that *Pythium splendens*, which in the Netherlands is found only in soil from green-houses, is also a heterothallic species. Tests with *Pythium intermedium* led to the observation that the formation of oogonia by this species is very dependent on temperature and medium. While oogonia are formed on a mixture of corn-meal agar and potato-carrot agar at 20 °C within 6 days, at 17.5 °C and 22 °C their formation is already greatly reduced. Numerous new strains were also isolated this year, especially from soil samples from Southern Flevoland. The soil of this polder seems to become steadily richer in *Pythium* species. From the last series of samples, thirteen different species of this genus were isolated. Further work was done on a manuscript about *Pythium* species with spiny oogonia. So far six of these species have been isolated.

Mrs. Hermanides-Nijhof and Mrs. van der Plaats-Niterink worked together to obtain fructification of Basidiomycetes in culture following a method described by Warcup and Talbot. The first series of tests, carried out with ten strains that had been in the collection for some time, were unsuccessful. A second series, with freshly isolated strains of *Rhizoctonia*-like fungi, resulted in two of the isolates forming basidia. Both of them probably belong to the genus *Ceratobasidium*. This work will be continued in 1970 as the formation of basidia is of great help in the identification of similar and otherwise sterile cultures.

Drs. H. A. van der Aa again collected, examined and isolated a number of new strains belonging to genera of the *Sphaeropsidales*. Some *Phyllosticta* macroconidial states could be cultivated from ascospores of *Guignardia* and *Botryosphaeria* species whose cycle was unknown until now. Various of these species which form microconidia are known in literature as *Asteromella*. Comparison of recent collections with herbarium type collections confirmed the conclusion that these spermogonial states definitely belong to another genus. Von Höhnelt's genus name *Leptodothiorella*, which has never been validly published, is the only one available for them.

Concurrently, species of the genera *Phoma*, *Ascochyta*, *Coniothyrium* and *Coleophoma* especially were more exhaustively studied. For the first time 5 critical species of *Coleophoma* of Mediterranean origin could be compared in vitro. Special attention was paid to the ascigerous and conidial states of *Didymella asphodeli*, *Guignardia cytisi* and *Glomerella tucumanensis*. The connexion of the last mentioned with *Colletotrichum graminicola* could be shown experimentally.

Dr. K. W. Gams went on with his study for a monograph on the *Cephalosporium*-like fungi. The chapters about the morphological divisions and the ecology, as well as a review of ascomycete genera with *Cephalosporium*-like conidial states are already written. The keys for the identification of genera and species are ready and also the descriptions and discussions of approximately 130 species which are spread over the genera *Acremonium*, *Verticillium*, *Aphanocladium*, *Emericellopsis* and others. About 30 of the taxa mentioned are treated as new.

Together with Mr. A. C. M. Hoozemans he examined the conidial states of some *Hypomyces* species, which had been isolated from toadstools. The species with dry phialospores with a broad attachment and with branched conidiophores were brought together, independently of the septation of the conidia, in the genus *Cladobotryum*. A manuscript about this investigation was prepared for publication.

In cooperation with Prof. K. H. Domsch (Braunschweig) the text of the book "Pilze aus Agrarböden" was completed. The preparation of the bibliography and the correction of the proofs of this publication, which has appeared in the meantime, consumed much time.

Dr. G. W. van Eijk carried out investigations in the field of isolation and characterization of fungal metabolites. After the acid production of some *Rhizopus oryzae* strains had been examined in 1967 from a chemotaxonomic viewpoint, the whole *Rhizopus* collection was included in this investigation in 1969. The results obtained will be compared with those from a morphological examination of these fungi.

Difficulty was encountered in the purification of the green pigment from a strain of *Roesleria pallida* on account of the labile character of the component. The best method of purification is being sought. The structure of the strongly fluorescent substance isolated from the fungus could not yet be determined. More material than the few milligrams isolated is necessary in order to carry out certain reactions. This is being worked on at the moment.

Several pigments were isolated from some *Streptosporangium* species. Some could be identified as phenazine derivatives. The structure of the others is being clarified.

In addition, cooperation was given in investigations being carried out in the Biochemical Laboratory of the State University in Utrecht into the lipid composition of fungi incubated under various conditions. These are being pursued.

Dr. J. A. von Arx finished the text of the publication "The genera of fungi in pure culture". Most of the illustrations were also made ready. He also pursued a study of the spore types occurring in fungi and gave a simple system for them.

YEAST DIVISION, DELFT

Miss Drs. W. Ch. Slooff completed the chapters about *Pityrosporum* and *Trigonopsis* for the revised edition of "The Yeasts". On the basis of the 42 strains studied, three species were recognized in *Pityrosporum*: *P. ovale*, *P. orbiculare* and *P. pachydermatis*. The latter name has replaced *P. canis*, which is a nomen nudum. Comparison of Gustafson's cultures with the original description of *P. pachydermatis* Weidman, and with the findings of Lodder (1931) proved that the reintroduction of the binomial *P. pachydermatis* was warranted, for those strains develop easily on regular laboratory media. Gustafson's strain 238s was designated as the neotype of *P. pachydermatis*.

On the subject *Lipomyces*, work was continued in cooperation with Drs. P. J. Nieuwdorp and Ir. P. Bos. Species in the genus were differentiated by the texture of the wall of the ascospores, the composition of the polysaccharides excreted in a liquid medium, and the pattern of carbon assimilation. Electron microscopic studies established three types of ascospore outer membranes: smooth; tuberculate; or provided with several ridges running longitudinally. The species *Zygotilomyces tetrasporus* and *Z. lactosus* were combined as *Lipomyces tetrasporus*.

An investigation is being carried out into an orange-red ballistosporegenous yeast which has symmetrical ballistospores. Such a form cannot be classified under the present system. It will be described in collaboration with Dr. F. T. Last who sent the strain for identification.

Drs. L. Rodrigues de Miranda spent the period from the end of July until mid October as a guest of Dr. C. F. Robinow, Dept. of Bacteriology, University of Western Ontario, London, Canada. He worked on the demonstration of nuclei and chromosomes of yeasts with the aid of staining techniques and the light microscope. An investigation was commenced into the mitosis in the genus *Lipomyces*, and this was later extended to include meiosis. This will be continued with the view to a possible application in the classification of asporogenous yeasts.

D. Yarrow studied the taxonomy of the genus *Saccharomyces* with the view to clarifying the position of those species which were found to have very unstable fermentation and assimilation characteristics (Scheda and Yarrow 1966 & 1968). The proton-magnetic-resonance spectra of the mannans (with the cooperation of Dr. J. F. T. Spencer), serology and nitrogen assimilation patterns are being determined. It has been found that the p.m.r. spectra of *Saccharomyces acetii* and *Saccharomyces ellipsoideus*, a species which can very easily be obtained from the former by a series of spontaneous mutations, are identical and that the strains behave very similarly serologically. Haploids derived from these two species conjugate and sporulate very readily.

J. A. VON ARX

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INSTITUTE OF ECOLOGICAL RESEARCH

PROGRESS REPORT 1969

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Population ecology	J. H. van Balen (head) J. A. L. Mertens (fysiology) H. N. Kluyver (guest worker)
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DEPARTMENT OF POPULATION ECOLOGY

I. *Population dynamics of the Great Tit (Parus major)*

The study of the population dynamics of the Great Tit, *Parus major*, is still the main research project. Routine observations on breeding density, clutch size and breeding success in several areas were continued.

a. Egg weight and clutch weight (J. H. van Balen)

Observations on the egg weight in the Great Tit were carried out in four areas. Apart from the comparative study in areas, which differ in food supply, it seemed valuable to study egg weight in relation to clutch size, and to the well-known decreasing trend in clutch size in the course of the breeding season. The results show that the weight of fresh eggs increases considerably during the breeding season. Among clutches laid during a short period there is a tendency for smaller clutches to contain larger eggs and vice versa.

The total weight of clutches decreases in the course of the breeding season due to a sharp decline in clutch size. Progressively less energy

is incorporated in eggs, but the available energy is divided among fewer and larger eggs as the season progresses. These tendencies may be related to the chances of survival for the nestlings of early and late broods.

The fresh weight of eggs from three rich oakwoods was considerably higher than that of eggs from a mixed wood, which is presumably poorer in food supply.

b. Sex ratio of nestlings (J. H. van Balen)

The sex ratio in the Great Tit has been discussed by many authors, but most of the results are biased by selective catching methods. Therefore it seemed important to determine the sex ratio exactly at an early moment, e.g. among nestlings. In 1968 a study of this subject was started. We used sex characters worked out by A. Dhondt (Gent). After much experience had been gained, it appeared possible to sex most of the nestlings. Captures in the autumn showed that ca. 90% of the nestlings was sexed correctly. Corrections – on the basis of recaptures – enabled us to determine the correct sex ratio of the nestlings, at an age of 16-18 days.

In the Hoge Veluwe area male nestlings predominated over females in a ratio of 60-40. The preponderance of males was partly due to differential mortality in the nest. In broods without nestling mortality there was still a slight, but insignificant majority of males.

The sex ratio of fledgling Great Tits therefore varies parallel with variations in mortality, which occur annually and locally.

c. Mortality, dispersal and moult in 1968/1969 (J. H. van Balen).

The study of mortality and dispersal of Great Tits in the Hoge Veluwe area was continued. Frequent recaptures of tits during winter and spring and the capture of the breeding population enabled us to compute the trends in numbers from summer 1968 to May 1969. In the summer of 1969, after the fledging of the 1969 generation, a new series of catches was started.

The results of the 1968/'69 season differed in several respects from those of the preceding year. In both years the numbers of first-year birds declined sharply between fledging and October, but in 1968 the decline was much more uniformly distributed over the period concerned. As in the preceding year changes in numbers between November and March were negligible.

The number of first-year immigrants entering the study area was small during the summer, and increased in August to a constant level. The high numbers in July and March, characteristic of the preceding season, were absent. The small number of immigrants in July may be due to the relatively high number of autochthonous first-year birds still present at that time.

The primary moult was studied in relation to the fledging date of the first-year birds. The information obtained over three years is summarized here. The start of moult is related to the date of fledging. Late-

fledged birds start moulting slightly earlier (in relation to the fledging date) than early-fledged birds. The rate of moult is slightly higher among the late birds. In general the earliest birds moult from the end of June to the end of August and the latest birds from the beginning of September to the middle of October.

By comparing the primary moult of immigrants to that of the autochthonous birds the approximate fledging date of the immigrants may be determined. Most of the immigrants in July-September appeared to be fledged from early broods.

The adult birds start moulting soon after or even slightly before the fledging date of their last brood. In general moult starts between early June and the middle of July, and ends between late August and late September. Females start later than males, and presumably have a higher rate of moulting.

d. Parasitism by *Protocalliphora* sp. (H. N. Kluyver, C. W. Eshuis-v.d. Voet)

The research on the biology of *Protocalliphora* sp. whose larvae parasitise on nestling Great Tits is now continued at the Laboratory of Entomology of the Agricultural University at Wageningen. It has been definitely established that larvae transferred from young tits to nestlings of other Passerines in the laboratory accept the latter as a host. We did not yet succeed, however, to make the flies lay eggs under laboratory conditions.

We continued our field observations and reached better insight in the biology of the larva and the fly. Most probably the fly is viviparous, as we never saw an egg in the observed nests. It is possible however that the eggs are laid at night and hatch in the early morning. Many times very young larvae were found in the afternoon. They feed only at night and probably only once in 24 hours, during less than half an hour. They stop feeding at the sixth day and begin to pupate, the pupa being completed at the eighth day. The pupa hatches after 6 to 24 days, the duration of this stage being temperature-dependent.

e. Regulation of numbers in the Vlieland population (H. N. Kluyver, J. H. van Balen)

Part of this study, comprising the years 1955-1967, was closed, and a publication is in preparation. This concerns the effect of the number of fledged young on the survival of both parents and young during autumn and winter.

Meanwhile the experimental study of the Great Tit population continued. The high breeding population of 1965, which was attributed to an amelioration in the habitat, was followed by a steady decline up till 1969, whereas the Blue Tit population is still increasing. The causes of these phenomena are yet obscure.

During 1967 and 1968 the reproduction has been artificially reduced to 3 fledglings per breeding pair. The effect of the reduction was roughly

equal to the reductions applied in earlier years, namely an increased survival of both adult and first-year tits.

II. *Energy balance of the Great Tit (J. A. L. Mertens)*

In the eco-physiological laboratory the heat production of resting adult Great Tits was measured by means of an indirect calorimetric method. The heat production increased linearly from about 0.3 kcal/hour at 16 °C to 0.8 kcal/hour at -30 °C.

Adult Great Tits, while asleep, can survive a temperature drop from +30 °C to -30 °C in 12 hours, at least in January and February. In March their resistance to low temperatures tends to decrease.

Under guidance of Professor Dr. C. T. de Wit (Agricultural University at Wageningen) we started to set up a simulation model for growth and survival chances of nestling Great Tits in dependence on food density and fluctuating temperatures.

A colorimetric method, to determine the uric acid content of bird excreta, was elaborated.

The temperatures in nine eggs of a Blue Tit clutch during incubation were measured simultaneously by means of very thin copper-constantan thermocouples. This investigation revealed, that the momentaneous internal egg temperature difference in broods comprising more than eight eggs can amount up to ten degrees centigrade.

III. *Energy balance of Starling nestlings (Sturnus vulgaris) (H. N. Kluyver, K. Westerterp)*

Nutrition, growth, respiration and excretion of young Starlings were studied in the field. Measurements of these four quantities in a natural situation cannot be made at the same nest. As the Starling is a colonial breeder and feeds collectively in the same pasture, it is possible to combine the observations on several nests. The size of the brood is important for the energy balance of the nestling. This is due to the greater heat loss per young in smaller broods.

The following methods were applied:

1. the food consumption was determined by taking food samples with the "collar method" and by automatic registration of the feeding frequency.
2. the growth of the nestlings was measured by weighing a number of broods periodically.
3. the production of faeces by the nestlings was measured by observations on the defaecation frequency and by weighing the faeces.
4. the energy content of food, nestlings and faeces was measured in a bomb calorimeter.
5. the heat production of the nestlings was measured in a respiration chamber at different temperatures.

This rather ambitious programme was executed within three months.

Only the samples which had to be examined in the bomb-calorimeter were frozen preliminary for later examination.

Though we realize that the quantities we tried to measure are not very accurate, we expect them to give an impression of the energy balance of a young Starling at nestling's age. Next year we hope to improve some of our measurements.

IV. *Ecology of the Collared Dove (Streptopelia decaocto)* (J. H. van Balen)

The study of the ecology of the Collared Dove was continued. Large numbers of doves, collected in the Harderwijk-Ermelo area, were examined. Sex and age of these birds were determined; body weight, wing length, wing moult and the condition of the reproductive organs were recorded, and the contents of crop and stomach were studied. At other localities large numbers of doves were caught for comparison.

During the period October-February the composition of the population was more or less constant, with a ratio of 53♂♂-47♀♀, and 62% first-year birds. During the breeding season it was impossible to obtain a sample unbiased as to sex ratio, due to differences in the daily feeding cycle of males and females. During October-November 1969 the sex ratio (52-48) was in good agreement with that of the preceding winter. From June the 1969 generation contributed to the population, with a share increasing from 23% (June) to 76% (September).

The extent of the breeding season may be determined from the conditions of testes and ovaries. Additional evidence is obtained from the condition of the crop wall, which secretes crop milk, an important source of food to young nestlings. The percentage of adult birds with a functioning crop wall was zero up till March. It increased from 10% in April to 52% in July, and decreased afterwards to 17% in October. Clearly July is the month when most parents have to feed nestlings, hence June is the peak month for egg production.

In the autumn doves were caught at Wageningen, at Deventer and on the island of Vlieland, in order to study local differences in the timing of primary moult. The results for Wageningen and Deventer were in good agreement with those from Ermelo, but birds from Vlieland were ca. 1 month behind the other groups. The causes of this phenomenon are still unknown.

Feeding trials with captive doves in outdoor conditions provided information on the preference for certain seeds, and on the amount of food consumed during different parts of the year. Daily food requirements vary from 12-15 grams (wet weight of seeds) in summer, to 18-22 grams in winter.

V. *Ecology of the Coot (Fulica atra)* (J. H. van Balen, J. Visser)

A research project on the ecology of the Coot, *Fulica atra*, which was started several years ago as a private study, was continued under

the Institute's supervision. The study takes place at the Westeinderplassen, in an area inhabited by ca. 150 pairs of Coots.

Clutch size, hatching success and survival of the young were studied. Mortality of the young occurs especially during the first three weeks of life, and roughly half of them reaches the age of two months.

The sex and age composition of the population was studied by frequent captures during most of the year. Catching techniques were developed and the possibilities of sexing coots were studied. The combination of several characters, such as wing length, body weight and dimensions of the frontal shield, enables us to sex most of the birds.

DEPARTMENT OF BIRD MIGRATION (VOGELTREKSTATION)

Homing experiments with Black-headed Gulls (Larus ridibundus) (A. C. Perdeck)

The experiments with Black-headed Gulls were finished this year. It was concluded that displacements to the north and the south at distances of 30 and 150 km. did not result in a clear homing tendency in the orientation cage. The results are given in the table below. The mean direction of each individual was calculated as the vector mean of the ranking of the 8 sectors at the bottom (according to the method described in the previous report). The table includes only observations with sunny wheather and sight on the landscape.

Distance	30 km.	150 km.
Number of observations	19 North + 19 South	17 North + 17 South
Direction of individual means:		
within "home" quadrant	7	13
right from "home" quadrant	11	7
left from "home" quadrant	11	8
opposite of "home" quadrant	9	6
P	>0.10	>0.10

Homing experiments with Greenfinches (Chloris chloris) (A. J. Cavé)

These experiments started in the winter 1967-1968. The aim was to obtain evidence if these birds show winterquarter-directed activity in Kramer-cages.

The Kramer-cages used have a flat bottom and a diameter of 60 cm. The bird walks in this cage on a white sheet of paper on which radially placed strips are mounted. These strips have a surface of felt soaked with ink. The footprints of the birds give a measure of the directions in which the bird is striving. The sheets of paper are changed each hour.

In the first winter we tried only a few individuals about 100 km NE and WSW of their winterquarter. No homing tendency was obtained. In the winter 1968-1969 these experiments were continued with a larger

number of individuals and at larger distances of home. The birds were captured in the garden of the institute. Each bird was tested some hours and they were given full sight of the landscape. Only hours with visible sun are included.

The direction in which the bird was striving is estimated by taking the vector mean of the directions of each registration hour (estimated by eye for the footprint pattern). Of the directions derived for the individual birds the vector mean is determined at each locality where they were tested after displacement. The individual choices are considered not to be in concordance with each other if this vector is too small to give a significant deviation from random with the Raleigh-test ($P \leq 0.05$).

The distances and directions of displacement and the results obtained are given in the table below.

Displacement of winterquarter	number of indi- viduals tested	mean direction
120 km W	12	random
230 km S	7	ENE (P 0.01)
260 km NE	4	NNE*
370 km ENE	19	random

* number tested too small for application of the Raleigh-test.

It is evident from the table that Greenfinches have no homing tendency in the Kramer-cages used. The experiment will not be continued.

Homing experiments with Starlings (A. J. Cavé)

The portable Kramer-cages used in the greenfinch-experiments are also used to estimate the choice of direction of Starlings after displacement during breeding. The same technique as described in the greenfinch-experiments is used. Only hours with visible sun are included. The birds were given full sight of the landscape. The birds were obtained from nestboxes placed at the "Hoge Veluwe" near Arnhem. The experiments were carried out during the breeding-season of 1969.

The birds were displaced to Schneverdingen (290 km. 65° from Arnhem). The direction of displacement is given as the number of degrees clockwise from North.

Also from these birds we obtained a number of registrations (of one hour each) of each individual bird. From these registrations a mean direction of each bird is obtained and the Raleigh-test is used again to conclude if the individual choices are in concordance ($P \leq 0.05$).

25 individuals were tested and their choices of direction were not significantly different from random. However, the direction of the mean vector (244°) was amazingly close to the home direction (245°). As a

consequence of the lack of agreement between the choices of direction, the latter result is not considered to be of significance.

Homing experiments with Pied Flycatchers (Ficedula hypoleuca) (A. J. Cavé)

Experiments about homing-activity in portable Kramer-cages to the nesting-place are also carried out with Pied Flycatchers. These experiments are identical to the starling-experiments.

The birds were taken from nestboxes in Warnsborn near Arnhem and displaced also to Schneverdingen (290 km, 65° from Arnhem).

It was not possible to test more than five birds. This number is too small for statistical analysis. However, as seen by eye their directions agreed rather well. Their mean direction was 114°, which is very different from the home-direction (245°).

With Starlings and Pied Flycatchers, experiments were also carried out near Dexheim, not far from Darmstadt (300 km, 155° from Arnhem).

This locality was very unsuitable since agricultural traffic disturbed the experiments continuously, especially south and west of the cages. The results are considered not to be reliable and not included in the conclusions.

Conclusions homing-experiments with portable Kramer-cages (A. J. Cavé, A. C. Perdeck)

There is no evident home-directed activity in these cages in experiments with Blackheaded Gulls, Greenfinches and Starlings. Also the results obtained with Pied Flycatchers are not promising. The experiments will not be continued.

Spontaneous migration activity of Chaffinches in the Kramer-cage (A. C. Perdeck)

6 adult females were tested in three successive situations, according to the following light conditions:

1. normal daily rythm.
2. daily rythm 6 hours advanced (dark at 12 h. noon, light at 12 h. midnight).
3. normal daily rythm.

It was assumed that a week in each situation is sufficient to reset the bird clock. The mean direction in each situation was calculated from 2 mornings with sunny weather, in the period from April 4th to May 8th. The results are given in the next table, where

A = mean direction in degrees before clock shifting

B = idem with advanced clock

C = idem with clock set back

(A + C) = idem of A and C together

Bird	A	B	C	B-A	(A+C)	B-(A+C)
1.	85	146	316	61	20	126
2.	4	227	75	223	35	192
3.	15	100	344	85	0	100
4.	62	150	36	88	49	101
5.	83	219	92	136	87	132
6.	349	349	265	0	307	42
Mean	40	168	18	99		115

Although the variation in direction between the individuals is considerable, the mean direction before and after the period of clock shifting is well in accordance with the migration direction. If this choice of direction is based on a sun compass orientation one should expect a 90° shift to the south in the clock shifted period. The mean difference between A and B is 99° . A better estimate is perhaps between (A+C) and B. Then the difference is 115° . These figures indicate that the sun compass is involved.

The 6 individuals were further tested continually, till mid October. They remained active during the whole period. In summer, however, the peak activity was not in the early morning but about at 10 a.m., with a smaller distinct peak in the late afternoon. The mean directions and the scatter are given in the next table (sunny conditions only).

Period	Mean direction (degrees)	Probability	Mean length of mean vector	Number of mornings
6-21 April	17 (NNE)	0.005	0.35	12
12 May-6 June	120	0.05-0.10	0.33	10
17 June-9 July	49	>0.10	0.27	11
5 Aug.-9 Sept.	42	>0.10	0.31	13
11 Sept.-15 Oct.	230 (SW)	<0.0001	0.36	17

It can be concluded:

1. during the migration periods there is a significant tendency to move into the migration direction appropriate to the season.
2. during summer there is no significant directional tendency.
3. the scatter of directions in each sample (one morning) is smaller during the migration periods than in between these.

Point 2 only holds true when all individuals are taken together. Each was oriented during the whole period, but they differed strongly in their directions, which moreover, changed in various ways.

Spontaneous migration activity of Chaffinches in portable Kramer-cages (A. J. Cavé)

The portable Kramer-cages with a diameter of 60 cm. are also used to determine the choice of direction during migration of the Chaffinch.

The birds used are obtained from Vlieland during the migration period considered.

The birds stayed for some days in the cages where they had full access of water and food and they were given full sight of the landscape. Testing took place in the first three hours after sunrise. The sheets of paper were changed each hour. The registrations obtained with visible sun of the same individual were combined by taking the mean vector direction of the directions derived of these separate registration hours.

The results of the spring are given in the table below.

Sex	Age	Directions
♀	after 1st year	74°
♀	„	15°
♀	1st year	84°
♀	„	3°
♀	„	28°
♀	„	35°
♂	after 1st year	301°
♂	„	97°
♂	1st year	211°
♂	„	137°

Mean direction 55°

The table shows that the individual choices are highly variable, but their mean direction is in rather good accordance with the direction to be expected in spring.

The results obtained in autumn are given in the table below.

Sex	Age	Directions	
♀	after 1st year	212°	Mean direction 187°
♀	„	216°	
♀	„	227°	
♀	„	314°	
♀	„	126°	
♀	„	150°	
♀	„	115°	
♀	„	297°	
♀	„	138°	
♀	„	208°	
♀	„	199°	
♀	1st year	141°	Mean direction 162°
♀	„	140°	
♀	„	330°	
♀	„	157°	
♀	„	292°	

(continued)

Sex	Age	Directions	
♂	after 1st year	41°	Mean direction 62°
♂	"	338°	
♂	"	138°	
♂	"	105°	
♂	1st year	108°	Mean direction 182°
♂	"	138°	
♂	"	209°	
♂	"	279°	

The southerly tendency is evident, with exception of the after 1st year males. This result suggests that these directions have indeed something to do with autumn-migration activity. They are, however, rather different from the expected standard direction, which is in the Netherlands distinctly more westerly. The reason for this discrepancy is unknown.

The field observations (A. C. Perdeck)

The observations on the standard direction of the Scandinavian Chaffinch were closed with additional observations on Helgoland, Cap Gris Nez and Stavoren.

A publication on this project will be published soon.

DEPARTMENT OF DISTRIBUTIONAL ECOLOGY

The field work of the department is centered in the IJsselmeerpolders, of which the newest polder Zuidelijk Flevoland was reclaimed in the spring of 1968, so that in 1969 we witnessed the second growing season. In this polder the colonization by higher plants and some insect groups is followed.

Vegetation development (J. v. d. Toorn)

Till the end of 1969 a total of 146 taxa of higher plants were found in Zuidelijk Flevoland (total number of the Dutch flora: 1357). 91% of these were found in a narrow zone inside the dikes, owing to the following properties of this zone:

1. more variation in habitat,
2. a more pronounced anthropogenic influence on seed dispersal,
3. a concentration of seeds by watercurrents before the reclamation.

Most of these species – with either anemochorous, hydrochorous or anthropochorous dissemination or a combination of these – reached the new land before it became dry. Only about 15 species were important for the general vegetation development.

Senecio congestus, one of the most dominant species, germinated in '68, was flowering during June '69 and died afterwards. The second generation

established itself only in lower parts of the polder, which were formerly covered with water, but dried up during July and August. Owing to the big seed output of the first generation, the density was in the range of 10-100 per m². In the rest of the polder the germination was hampered either by shortage of soil moisture or by competition of perennial plants.

In the spring *Ranunculus sceleratus* was co-dominant with *Senecio congestus*. Locally the density of the 2nd generation, flowering in May '69, was 10-20 individuals per m². These high densities were found at the edges of rainwater pools, where seeds of the first generation (grown in '68) were concentrated as a result of hydrochorous dissemination. The third generation plants, germinated in May '69 developed poorly or died, mainly due to a strong competition for light by *Phragmites communis*, although shortage of soil moisture may have been of influence too.

The artificially sown *Phragmites communis*, which in spite of a high density, had a low coverage in '68, showed a strong vegetative development during 1969, which resulted in a coverage of about 60-100%, at the end of the growing season.

The vegetation development was studied by describing and photographing 32 vegetation plots periodically. In 4 plots, representative for a large part of the polder, the mean number of plant species increased gradually from 4 (June '68) to 15 per 25 m² (August '69), of which 5 were perennial. This increase was caused during 1968 by a retarded germination of seeds present in the soil and during 1969 by the progeny from (relatively rare) species outside the plots.

In the same period the number of plant individuals increased from c. 50 tot c. 1100 per 25 m², with a top in June '69 (c. 1600 per 25 m²). This quick increase was mainly caused by ephemeral and annual species, especially by *Ranunculus sceleratus*, owing to its short life cycle (2-3 months) and its high seed output (400.000-1.000.000 seeds per plant).

The plant cover in the above mentioned period increased from c. 5 to c. 80%. Up till June '69 ephemeral and annual species dominated but afterwards perennials took over.

Carabid beetles (J. Haeck)

The reclamation of the IJsselmeerpolders may be regarded as one large zoogeographical experiment to investigate the immigration into a large virgin area within the existing range of land-animals. With this experiment it will for instance be possible to test LINDROTH's hypothesis that in wing-dimorphic Carabid beetles the proportion of brachypterous individuals in a population is correlated with its age.

The beetles are sampled by pitfall-trapping in different places in the new polders and the border area, and by intercepting flying animals in window traps. Most of the material collected in 1968 is now identified. In 1968 in Zuidelijk Flevoland over 2000 Carabid beetles were caught in the pitfall-traps and about 450 individuals in the window traps. Of the

56 species found, 44 were monomorphic macropterous, 12 dimorphic and 0 monomorphic brachypterous. The dimorphic and constantly macropterous species were found in about the same proportion as on the mainland. From this we may conclude that the chances for reaching the new land are not smaller in dimorphic species, but much smaller in brachypterous than in macropterous species.

In Zuidelijk Flevoland only along the dike a very few brachypterous individuals of dimorphic species were caught: 4 specimens of *Carabus granulatus* L. and 1 specimen of *Trechus obtusus* Er. Of *Trechus quadristriatus* Schrk., which is a sibling species of *Trechus obtusus*, but monomorphic macropterous, many individuals were caught in the window traps and in funnel traps in the middle of the polder. (This statement gives a correction on data in the progress report of 1968, due to a misidentification of this species). In the polder Oostelijk Flevoland, which ran dry in 1957, the proportion of winged individuals in the populations of dimorphic species is also very high in comparison to the situation on the old mainland. The winged individuals are obviously of paramount importance in the establishment of new populations of these dimorphic species.

The species found in Zuidelijk en Oostelijk Flevoland are predominantly originating from unstable (riparian or ruderal) environments. In these environments the frequency of constantly macropterous species as compared to dimorphic species is higher than in more stable environments (woods, heaths) (cf. DEN BOER, *Oecologia* 4, 1970). This suggests that in an unstable environment extinction of populations is of too frequent occurrence to allow the spread of much "shortwingedness".

Of the samples of Zuidelijk Flevoland from 1969 a small part is identified. The number of species caught in one place increased: in the window-traps 1 km off the dike in the polder 17 species were caught in 1968, 24 in 1969; in the pitfall-traps in the middle of the polder 13 species were caught in 1968, 28 in 1969. Sometimes within a week we got a catch of 30 individuals of one species (*Bembidion varium* Oliv.) in one window trap. From this we may speculate on the number of individuals of this species into the polder: over a cross section of 20 km of the polder and on a height of 1.00-150 m above the surface this would mean a total of 600.000 in one week.

These results will be more extensively reported in a paper appearing in *Belmontia* (1970).

Phytophagous insects on reed and willows (J. H. Mook)

During the summer of 1969 again a number of insect species living on Common Reed (*Phragmites communis*) and willows (*Salix* sp.) were sampled along a transect running from SW to NE through the polder Zuidelijk Flevoland.

The aphid *Hyalopteris pruni* was sampled on reed in June, just after

the immigration of alate females originating from *Prunus*, a plant genus that does not yet occur in the polder inside the dikes. These aphids thus have to migrate from the old land or from the polder Oostelijk Flevoland. The mean number of alate females per reed shoot was larger in the SW than in the NE-part of the transect. Furthermore it was remarkable that, although the decline from SW-NE was found both in wet en dry places, the number of alatae in wet places was systematically higher than in dry places. There were also differences in the subsequent growth of populations in dry and wet places.

So far, the investigation was restricted to the recording of the numbers of aphids found in different places and in different environmental situations. Its aim, however, is to find the causes for this distribution. Because the migration of *Hualopteris* to the polder is a yearly phenomenon (as long as no *Prunus* is planted inside the dikes), it will be possible to test by field experiments the influence of factors like distance from the old land, climatic differences between sample sites and preference of the aphids for reed growing under different water regimes.

On the willow species *Salix alba*, *S. triandra* and *S. viminalis* the distribution of galls and mines was sampled along the transect in September and October. For the different species different distributions along the transect were found, suggesting that some species migrate rapidly (*Pontania proxima*, a Tenthredinid wasp on *Salix alba*, as well as the gall midge *Giraudiella inclusa* on reed), some more slowly (the gall midge *Rhabdophaga marginemtorquens* on *Salix viminalis*) and others very slowly (e.g. the gall midge *Rhabdophaga heterobia* on *Salix triandra*). The distribution of the curculionid beetle *Rhynchaenus populi*, mining the leaves of *Salix triandra* suggests that very few females of these beetles are migrating over long distances in a period before egg-laying, because although the species is common outside the polder, very few willows were found to be attacked along the whole transect, but those willows that were attacked were more or less heavily infested.

As in the case of the distribution of aphids on reed it will be necessary to distinguish between influences of distance and of physiological and genetical differences in the willows. To investigate the influence of soil composition and of water regime on insect attack, a field experiment is in progress in the polder Oostelijk Flevoland in which genetically homogeneous plant material is growing along a gradient ranging from dry sand to moist clay.

BIOLOGICAL STATION "WEEVERS' DUIN"

Vegetation research (D. van der Laan)

One of the aspects of the research on vegetation of the dune area on Voorne is the typology of the dune slack vegetation as a base for further ecological work.

The aim of this study is to arrive at a system in which stands out the relationship vegetation-environment and the differences respectively similarities among the elements of the system themselves.

In connection with the method used up to now it is necessary to be familiar with a number of important environmental factors of the sample plots of which vegetation analyses are used.

These factors are the groundwater regime as well as some chemical- and physical soil factors.

From previous studies it appeared that the water table highly affects the floristic composition. Within a hydrologically homogeneous area water table and vegetation show an exceptionally high correlation.

The situation is more complex if the area in which the vegetation analyses are collected is not hydrologically homogeneous. This is the case inside given slacks as well as in the slacks on Voorne as a whole. Thus the groundwater table at a given time is not a representative measure for the groundwater regime as a whole, suitable to compare different sites. However, as the typology is based on important ecological factors, one of which is the groundwater regime., it is indispensable to have a measure for this factor that can be used over the area as a whole.

An index for the groundwater regime was found by working up a representative number (40) of the vegetation analyses taken. These analyses were subjected to a three-dimensional ordination. The correlation is calculated between the position of the vegetation records on the X-, Y- and Z-axis and several characteristics of the water regime. The Y- and Z-axis did not present any significant correlation with the water table and are left aside here.

The characteristics of the water regime considered and the correlation coefficients belonging to them are:

1. Average water level throughout the year	0.68
2. Average water level during the vegetation period	0.80
3. Maximum water level	0.24
4. Minimum water level	0.55
5. Total inundation period	0.63
6. Inundation period in spring	0.54
7. Inundation period during the vegetation period	0.64
8. Height of site above mean sea level	0.53

Because the water table appeared to be of such an importance much attention is given to the network of water table pipes, which is still being enlarged.

Besides the water table, the content of organic matter and the soil structure are considered as major factors. Other soil characteristics like the content of Cl-ions, phosphates, CaCO_3 and pH value are involved in the study.

An experimental approach will be used in examining the influence of

the soil structure by increasing in artificial way the compactness of the soil. A given technique to quantify the soil compaction will be studied.

Micrometeorological work. (Ph. Stoutjesdijk)

The problem of heat and water relations of leaves and other evaporating surfaces was further analysed and the following scheme of the mutual relations of surface properties, radiation, wind velocity and air humidity was arrived at

1. Surface wet, no radiation.

The simplest case. The temperature of the surface is fundamentally independent of wind force and the same as the wetbulb temperature of the psychrometer. Heat transfer and evaporation are coupled, as both take place through a boundary layer of still air. Evaporation intensity can be expected to be proportional to the square root of wind velocity. Wet mosses fall under this scheme and possibly some amphibians and slugs.

2. Surface wet, strong solar radiation.

With increasing wind speed, temperature sinks, evaporation increases less than in case (1). With strong wind this case approaches to (1).

3. Surface partially wet, no radiation.

The term "partially wet" is used here for a surface which has a higher resistance against water loss than a free water surface.

A leaf of a higher plant has according to van den Honert and others an internal as well as an external resistance against water loss, the latter being the same as that of a free water surface. Many membranes of mushrooms, amphibians, arthropods will fall under this scheme. Approximately a proportionality of water loss with the saturation deficit of the air can be expected. Increase of air movement will result in increase of water loss and a fall of temperature, but the higher the ratio internal resistance/external resistance the smaller the influence of wind will be.

4. Partially wet surface strong solar radiation.

The most complicated case of which transpiring leaves are the main representative but, of course, other partially permeable membranes fall under this scheme.

The following subdivision can be made:

a. The internal resistance of the leaf is many times (say: 10 or more to fix thoughts) as high as the external. Transpiration is relatively weak. Leaf temperature is considerably above air temperature as long as air movement is weak. Wind decreases the external resistance but owing to the much higher internal resistance the total resistance decreases only slightly, leaf temperature decreases considerably, hence the vapour-pressure difference leaf-air decreases and transpiration as well. The effect will be stronger the more humid the air is.

Heat and vapour transfer are not directly coupled in this case because for heat transfer only the external resistance is of importance for water vapour transfer the total resistance is decisive.

b. The internal resistance of the leaf is only a few times higher than the external resistance (we found in our measurements a lowest value of about 2 for the ratio internal/external resistance).

When the leaf is warmer than the air, leaf temperature will always decrease when air movement increases. Transpiration can either become somewhat stronger or somewhat weaker. Under temperate conditions usually a small decrease of transpiration by wind can be expected.

When the leaf is cooler than the air, as can be the case when the air is dry, transpiration always will increase (but never strongly) with increasing wind, but temperature can both rise or fall a little. For concrete cases it can always be calculated what is to be expected.

Of course these considerations are only valid when no physiological reactions occur like opening and closing of stomata or changes of permeability or when there is a pumping effect of wind. Working out the consequences of this model make it possible however to test its validity.

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Ecophysiological investigations of coastal plants: growth-physiology, salt-tolerance and mineral nutrition (A. H. J. Freijssen).

In 1969 the scientific programme of the Biological Station "Weevers' Duin" was enlarged with a new field of ecological research aimed at the growth-physiology, the salt-tolerance and the mineral nutrition of coastal plants. In the greenhouse germination, growth rate and shoot/root ratio are investigated. In the laboratory a chemical analysis of the dried plant material is carried out. In this the concentrations of the major ions and organic N are determined. On the basis of such analyses a picture of the ionic balance of each plant is formed. The results of the ecophysiological experiments are related to the ecological preference of the plants investigated. The following species were studied: *Centaurium littorale* (Turner) Gilmour; *Cynoglossum officinale* L., *Festuca rubra* L., ssp. *arenaria* (O.) Richter and ssp. *litoralis* (G.M.) Auquier. In the Netherlands all these species are typical for coastal habitats. Below some main results from the experiments are mentioned together with their ecological importance.

Centaurium littorale

Centaurium littorale was grown in a sand culture under various nutritional conditions and at different degrees of salinity. With a sufficient supply of mineral nutrients rosette-plants exhibited a very slow dry matter

production. The relative growth rate was only $0,02 \text{ mg.mg}^{-1}.\text{day}^{-1}$. It is generally accepted that a low growth rate is an important means for a plant to cope with an oligotrophic environment. In all experiments plants of *Centaurium littorale* possessed a low shoot/root ratio averaging 0,8. On the one side this low ratio explains at least partly the low growth rate, on the other hand a relatively extensive root system may be considered as an adaptation to poor and/or dry soils. High degrees of salinity in the culture medium caused high levels of Cl' and Na^+ to occur in the dried tissue material, without further effects on the ionic balance. The high concentrations of Cl' and Na^+ raise osmotic values which enable the plant to grow normally up to a salinity degree of 7 atm. Apparently the uptake of other minerals especially K^+ is not hindered by greater quantities of NaCl . This salt tolerance and the above mentioned characteristics of the growth physiology of *Centaurium littorale* are considered to be adaptations to its natural environment. This is the bare soil of dune slack margins and beach plains, consisting of coarse sand. This dune sand is highly deficient in plant nutrients and rather rich in NaCl .

Cynoglossum officinale

Cynoglossum officinale was grown on a Hoagland nutrient solution. Very young rosette-plants attained a relative growth rate up to $0.12 \text{ mg.mg}^{-1}.\text{day}^{-1}$. This growth rate is about the same as those of *Senecio congestus* and *Lolium perenne*. Both these species occur on rich soils. The shoot/root ratio of *Cynoglossum officinale* appeared to be 1,8. With respect to the ionic composition of the plant material the following values (me. per kg dry matter) are notable: $\text{N-org.} = 3500$; $\text{NO}_3^- = 500$; $\text{K}^+ = 1500$. These quantities correspond with those of agricultural crops, i.e. plants with a high uptake of mineral nutrients. These data suggest that *Cynoglossum officinale* is adapted to superfluous nutrient conditions. This seems to be in contradiction with the distribution of *Cynoglossum officinale* in sandy dune areas, which are mostly oligotrophic, as already stated above. However, within these dune areas *Cynoglossum officinale* has an obvious preference for disturbed places, where the soil is enriched by the activity of man or animals.

Festuca rubra (Miss E. Heeres)

The influence of salinity on germination and growth was studied in a comparative experiment with *Festuca rubra* ssp. *arenaria* and ssp. *litoralis*. The former appeared to possess a rather wide tolerance, the latter attained still 1/3 of its maximal yield at 1% Cl' . The uptake of Cl' was very high in both subspecies. These differences in salt tolerance correspond with differences in habitat. *Festuca rubra* ssp. *arenaria* occurs in the outer dunes where the soil moisture is brackish as a consequence of salt spray. *Festuca rubra* ssp. *litoralis* is typical for the spring tide level on the salt marsh.

Food differentiation in the moth genus Eupithecia (R. de Jong)

In the dunes of Voorne 21 species of the moth genus *Eupithecia* have been observed. The work starts from the idea that in the course of their evolution these species not only became differentiated morphologically, but also ecologically, so that closely connected species can occur side by side in the same area. The investigation is concentrated on the relation (if any) between the occurrence of so many more or less closely connected species in a relative small area and the differentiation in food choice.

Although a species can only be resident in an area, if there is food for both larvae and imagines, the food demand of the imagines is not taken into account, assuming that the supply of food for the imagines is not a limiting factor in the occurrence of the species in the dunes of Voorne. So the following concerns the larval stage.

Among the 21 species 7 species are monophagous and as each of these species has his own plant species to feed on, it is clear that the differentiation in food choice has led to the possibility of their occurrence in the same area.

The other (14) species show a considerable variation in polyphagy, some feeding on two or three, others on many plant species. Although there is a differentiation in food choice, the food demands partly overlap. Without other factors working this could lead to a strong competition. So the study is greatly devoted to the questions: does competition exist between the polyphagous species and what are the factors limiting or prohibiting competition?

The existence of such factors can be illustrated by the following example. On *Senecio* three species (*absinthiata*, *subfuscata* and *centaureata*) have been found. About 75% of the larvae found on *Senecio* belonged to *absinthiata*. This species feed, apart from *Senecio*, on *Eupatorium* and, incidentally, on *Achillea*. The other two species have a much more differentiated diet, especially *subfuscata*. So their relatively low numbers on *Senecio* do not a priori mean that they are scarcer than *absinthiata*.

Both *absinthiata* and *centaureata* feed on the flowers and immature seeds. To reach the seeds they normally perforate the involucre bracts. Although *subfuscata* too feeds on the flowers, it does not perforate the involucre bracts to reach the seeds, but only grazes on the disk-flowers. A further differentiation is found in the habitats: *absinthiata* and *subfuscata* love sheltered places, while *centaureata* can be found in more open places, even on the exposed top of a dike without any shelter. This makes it understandable that although all three species feed on *Senecio jacobaea*, only *absinthiata* and *subfuscata* have been found on *Senecio erucifolius*, in the dunes of Voorne a plant of somewhat sheltered places.

Further factors limiting a possible competition are predation and parasitism. Predation has not yet been studied sufficiently to allow conclusions, but it is clear that parasitism greatly influences the numbers of individuals. Of the *absinthiata* larvae at least 60% was attacked by a *Chalcidid*

wasp and about 75% of the infected larvae died before they were two weeks old. Thus, about 45% of the larvae was removed from the population before taking a considerable part of the food.

Although these factors unmistakably limit a possible competition, the fact that sometimes two species simultaneously feed on the same individual plant clearly shows that competition can exist. To study competition larvae are reared in different densities and species combinations. Up to now there are no reasons to believe that competition plays an important role in the field.

Probably the larvae remain on the plant where they hatched from the egg, so the distribution and the density of the larvae are greatly determined by the oviposition of the female moth. The preference of the female moth for certain plant species to lay the eggs and the number of eggs laid on one plant and in one night are studied by cage experiments, where fertilized female moths can choose out of different plant species. Mainly reared moths have been used to check the possible preference for plant species where they fed on as larvae. The results do not show such preference, but further experiments are needed.

W. H. VAN DOBBEN

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Due to rough weather on some days the programme could not be carried out.

Changes in silicate, phosphate and nitrogen compounds:

Due to the high silicate content of the polder water the winter concentration of $\text{SiO}_2\text{-Si}$ is about 5 mg/l. This concentration falls during spring to 0.1 mg/l. The decrease seems to be correlated with the development of the diatoms. The lowest silicate concentration was reached at the end of May in 1969; and in early May in 1968. Accordingly, the spring diatom maximum (see below) in 1969 occurred one month later than in 1968.

In the wet summer of 1968 the inflow of polderwater caused a gradual increase of silicate from 15 September onwards. In 1969 only a small increase of silicate was found in September (± 1 mg/l), gradually decreasing till the end of October, when the polderwater inflow started again. Accordingly the autumn diatom bloom was much smaller in 1969 than in 1968.

Inorganic phosphate concentration fell in May 1968 from 0.3 mg/l to 0.05 mg/l. ($\text{PO}_4\text{-P}$). In 1969 the concentration decreased nearly linearly from February (0.4 mg/l of $\text{PO}_4\text{-P}$) till the middle of August (0.04 mg/l of $\text{PO}_4\text{-P}$). The total phosphate followed the same pattern as inorganic phosphate in both years. Particulate phosphate was about 0.150 mg/l part-P in January-February and decreased to 0.08 mg/l in July. Unfortunately no distinction can be made between cellular and dead particular phosphate (bottom-material). The dissolved phosphates are partly inorganic and partly organic phosphates. The latter hydrolyse slowly in acid conditions to inorganic phosphate, so that for the determination an extraction procedure must be used.

IJsselmeer water brings nitrate and polderwater brings ammonia into the lake water up to 1-1.5 mg/l $\text{NO}_3\text{-N}$ and up to 1.5-2 mg/l $\text{NH}_3\text{-N}$. Particulate nitrogen was about 1 mg/l in the first half of the year. Unfortunately the values for the second half year are missing. The oxidation of ammonia to nitrite almost stops during the winter. Nitrite concentrations are varying rapidly between 1 and 20 $\mu\text{g/l}$ of $\text{NO}_2\text{-N}$. There is no obvious pattern.

Phytoplankton

Miss de Bruyn continued the phytoplankton counts (by the Utermöhl method), taking samples each fortnight at three sampling stations in the lake and one in the Broeresloot. As in 1968 the most abundant algae were *Scenedesmus* (div. spec.) and *Melosira*. This diatom reached a higher number of threads than in 1968. This year however, the threads were 2 à 4 cells long (8 à 10 in 1968). The spring bloom was about one month later than in 1968; there was no autumn bloom. The numbers of the *Cryptophyceae* (*Cryptomonas*, *Chroomonas*) and *Chlamydomonas* were higher than in 1968 both in the lake and in the Broeresloot. The plankton data are summarized in table 2.

Drs. Kraayeveld obtained cultures of *Melosira*, *Diatoma* and *Asterionella* from Tjeukemeerwater. The cultures, which are now kept at 12 hr light

Organism	1968		1969	
	period	cells max/ml	period	cells max/ml
<i>Melosira</i> div. spec.	14/2-24/4	9000	1/4-30/7	13.000 (7/7)
	11/9-15/10	3.600-8100	September	1.500 (24/9)
<i>Oscillatoria</i>	10/4-3/7	max. 80% of total	—	—
<i>Scenedesmus</i> div-spec	29/4-5/6	12.000 (22/5)	whole year	24.000 (15/4)
	1/8-9/10	48.000 (24/9)		28.000 (5/11)
Centric diatoms	27/3-1/5	800 (20/4)	1/4-20/10	1.600 (2/5 and 21/5).
(<i>Actinocyclus</i> <i>normannii</i>)				
<i>Stephanodiscus astrea</i>		400 (24/9)		
<i>Cyclotella</i> spec. div.)				
<i>Chlamydomonas</i>			whole year	100-800 (3/7)
<i>Cryptophyceae</i>			1/5-20/10	400-1100 (21/5) 600 (10/9)

Table 2: Cell numbers of the most important algae in Tjeukemeer.

dark rhythm and at 10 °C, are not yet bacteria free, although bacterial growth is suppressed.

Antibiotics and U.V. light will be applied furthermore for this purpose. With these cultures Drs. Kraayeveld will try to measure the growth rates under different light-intensities, nutrient concentrations and at different temperatures in order to explain the cause of the spring or autumn maxima of these populations.

Looking for a suitable natural model for studying algal periodicity Mr. H. L. Hoogveld counted phytoplankton in two small lakes near Tjeukemeer in the Netherlands. Interesting growth and dying phenomena of *Chrysophyceae* were observed, especially involving *Synura* spec., *Mallo-monas* spec., and *Kephyrion* spec.

Primary production

As the light penetration in the lake water is poor ($Z_{0,d.} = 4$ to 10 cm in 1969) and the weather often rough the determination of the primary production with suspended bottles is unreliable. Long glass tubes (2.5, 5.0 or 7.5 dm) long, Ø 5 cm) were used for the C^{14} method. When the photosynthetic layer is no deeper than 2.5 dm (as often is the case) the activity per litre in the 5.0 dm tube and in the 7.5 dm tube are 50% and 33% of the activity in the 2.5 dm tube. The total activity is calculated as $2.5 \times (\text{mg/l CO}_2/\text{hr})_{2.5 \text{ dm}} + 5 \times (\text{mg/l CO}_2/\text{hr})_{5.0 \text{ dm}}$ or $7.5 \times (\text{mg/l CO}_2/\text{hr})_{7.5 \text{ dm}}$.

From the ratio's in the 2.5 dm and 5.0 dm tubes the total photosynthesis per dm² can be calculated.

The O₂-production and C^{14} uptake are measured in Winkler bottles at 10 cm depth. From these values the A.Q. is calculated. From the ^{14}C tubes

the production may be estimated as ± 150 (winter)-1500 (summer) mg of C per m² per day. Oxygen consumption during the warm period is about 2.5 g/m² of O₂ per 8 hr. No distinction can yet be made between oxidation of (allochthonous) organic material and algal respiration.

In table 3 the following data are summarized:

- A. g/m³ of CO₂/hr at point I in tubes.
- B. g/m³ of CO₂/hr at point I and g/m³ of O₂/hr at point I, II, III.
- C. A.Q.
- D. Chlorophyll
- E. PO₄-P and Tot-P concentrations and
- F. g/m² of CO₂/day, calculated as

$$(\text{mg/l of CO}_2 \text{ hr}^{-1})_{\text{a dm tube}} \times \text{a} \times \frac{\text{Lux hr/day}}{\text{lux hr/obs. period}}$$

Chlorophyll is calculated as $13.9 \times D_{665}$ (IBP-Manual 12).

The ratio of the extinction of the unacidified chlorophyll extract over that of the acidified extract varied around 1.5 ± 0.1 . No correction for phaeophytin was made.

Mrs. N. F. Frederik finished her study of degradation breakdown products of chloroplast pigments. Using thin layer chromatography she found phaeophytins and degradation breakdown products in the phytoplankton of the Tjeukemeer.

Her results concerning induction of degradation of chloroplast pigments in *Scenedesmus obliquus* by chloroform treatment were in agreement with earlier data obtained in this laboratory. It can be concluded that phaeophytin *a* and *b*, occurring in natural waters, can be induced by chloroform treatment of intact *Scenedesmus obliquus* cells in the dark.

Mr. T. van 't Hof continued this study of the phytoplankton pigments. The detection method-thin layer chromatography- will be improved, whereby it will be attempted to characterize the predominantly occurring green degradation product.

Drs. de Haan, who works on the humus compounds developed a new extraction method. Easily occurring chemical alterations of humus compounds, caused by pH variations, are prevented by maintaining the pH at 7.0 during the whole procedure.

Humus compounds, extracted in this way from Tjeukemeer, seem to be of allochthonous nature. They make up the largest part of the total amount of organic compounds in the lake. The extract has been separated by Sephadex-gel filtration into three main fractions. Their molecular weights range from less than 500 to above 50.000. Optical properties of the fractions are being studied as well as iron and phosphate binding capacities. The relative amount of the fractions in the extract is dependent

DATE	METHOD	PHOTOSYNTHESIS					A.Q.	Chlorophyll PO ₄ -P Tot-P		
				g.m ³ ·hr ⁻¹ .		g/m ² /day				
		I	I	II	III					
		¹⁴ CO ₂		O ₂		CO ₂		mg/m ³ ²⁾	mg/m ³ ²⁾	
5-2-1969	25 cm	0.12								
	75 cm	0.04								
	Wi i.s.	0.19	0.11				1.3	17	340	450
2-4-1969	25 cm	2.13				5.0		35-50	350	400
	75 cm	0.92				6.5			100	
	Wi i.s.	2.86								
6-6-1969	25 cm	1.80				6.1		55-95	90	300
	75 cm ³⁾	0.54				5.5				
	30 cm st.	1.40				5.6				
	Wi i.s.	2.27	1.06	0.98	1.08	1.5				
16-6-1969	25 cm	1.32				4.3		30-60	70-100	300
	75 cm	0.61				6.0				
	Wi i.s.	1.90	1.94	1.36	1.59		0.72			
2-7-1969	25 cm	1.15				3.4		20-45	80	200-250
	75 cm	0.59				5.4				
	Wi i.s.	0.64	0.90	1.19	0.86		0.52			
16-7-1969	25 cm	1.15				3.5		15-35	60-80	150
	75 cm	0.57				5.2				
	Wi i.s.	1.28	0.67				1.4			
30-7-1969	25 cm	0.35				0.9		20-30	50	125
	75 cm	0.12				1.0				
	Wi i.s.	0.53	0.32	0.32	0.28		1.2			
13-8-1969	25 cm	1.39				3.5		20	40	100
	50 cm	1.14				5.7				
	75 cm	1.00				7.5				
	Wi i.s.	0.97	0.63	0.54	0.35		1.1			
10-9-1969	25 cm	0.67				1.7		15-25	150	250
	50 cm	0.35				1.8				
	Wi i.s.	0.75	0.22	0.29	0.73		2.5			
8-10-1969	25 cm	2.34				5.2		10-20	100	100-150
	25 cm ²⁾ st.	2.26				5.1				
	50 cm	2.15				9.7				
	Wi i.s.	3.44	0.39		0.57		6.44			
5-11-1969	25 cm	0.47				0.7		30-40	200	450
	25 cm ³⁾ st.	0.64				0.9				
	50 cm	0.27				0.8				
	Wi i.s.	1.21	0.62	0.28	0.46		1.4			
2-12-1969	25 cm	0.17				0.25		15	150-200	200-350
	25 cm ³⁾ st.	0.19				0.28				
	50 cm	0.14				0.42				
	75 cm	0.09				0.40				
	Wi i.s.	0.27	0.17	0.12	0.34		1.2			

¹⁾ Long tubes or Winkler bottles in situ

²⁾ Mean for points I, II, III estimated from the year curve

If a range is indicated one point — in most cases I — is lower than the others.

³⁾ Closed 25 cm tubes, but with stopper.

on the season. As soon as "polder water" comes into the lake (autumn and winter) the amount of the highest molecular weight fraction ($M.G. > 50,000$) increases, as does the amount of the smallest molecular weight fraction ($M.G. \leq 500$). The amount of the fraction in between ($500 \leq M.G. \leq 50,000$) does not alter much during the year.

Sephadex elution patterns of the same extract seem to be pH dependent. The molecular size increases as pH of the eluant is increased. An increase in colour of the extract with increasing pH was also found. Both findings are attributed to increased functional group ionization. Drs. de Haan started to study whether the humus compounds in Tjeukemeer can be degraded by micro-organisms in the lake. If this is the case what is then the role of this bio-degradation in the carbon cycle in Tjeukemeer.

Dr. Moed affiliated with the Physical Oceanographic Institute of the Nova University in Fort Lauderdale (Florida) for the period of one year, performed investigations concerning yellow water soluble compounds.

From exudates of Sargassum weed, using paperchromatography, 2 yellowish-brown bands could be obtained which responded positively to the vanillin test for phloroglucinol. Prolonged incubation of Sargassum weed showed yellow precipitates. It is doubted whether chloroplast pigments of marine algae, as literature data suggest, can give rise to yellow soluble compounds.

Study of yellow canalwater from the Everglades which partly enters into the Ocean at Fort Lauderdale, showed that at least four fractions can be isolated, using Al_2O_3 columnchromatography. By measuring fluorescence spectra it was found that one fraction showed an intense scattering effect for the wavelength at which it was excited ($375 m\mu$). Performing gelfiltration a better impression of the heterogeneity of the yellow material was obtained.

As yellow material showed an affinity for beach sand it was tried to release such compounds from beach sand. UV and fluorescence spectra of yellow material released with EDTA were very similar to those for canalwater.

UV spectrum of yellow material released with HCl showed a plateau at $260 m\mu$. For degradation studies alkaline fusion as well as treatment with $NaIO_4$ seems to be promising.

As a possible biological source, evidence has shown that saw-grass (*Mariscus jamacensis*) must be considered.

The zooplankton (*Copepods* and *Cladocera*) was studied by Drs. Vijverberg. All main species, including the copepodite stages of the *Copepods*, were determined on to the species. The most abundant species were: *Acanthocyclops robustus* Sars, *Mesocyclops leuckarti* Claus, *Bosmina coregani* Baird, *Bosmina longirostris* Müller and *Daphnia cucullata* Sars.

In most species there was a marked difference in the annual abundance.

The horizontal and vertical distribution was very much influenced by the water turbulence.

Biomass was estimated by using the volumetric method of Lohman (1908). The zooplankton was measured and the volumes estimated by reducing the body shapes to single geometric bodies. The biomass was calculated for each copepod stage and for 21 different size classes in *Cladocera*. Biomass was expressed in g/m² lake surface. Total biomass was highest in summer (5.9 g/m² and 9.6 g/m²) and lowest in winter (approximately 0.1 g/m²). The biomass in spring and autumn was approximately 3.1 g/m². Using Winbergs P/B values (Winberg 1968) the production in the vegetative season was estimated to be 650 kg/ha in 1968 and 950 kg/ha in 1969.

Drs. Peeters orientated himself about possibilities for research of population organisms and productivity of some groups of the Tjeukemeer fauna. Furthermore he developed a documentation-system for limnological and ecological literature.

Mr. Beattie continued his studies of the Chironomid fauna of Tjeukemeer. In the mud area all species were accurately recorded throughout the whole year. The main standing crop biomass of this area was made up by *Chironomus anthracinus* and *Clyptotendipes paripes*, having a cotagious distribution from development of isolated egg-masses and migration of 4th instars from the surrounding high-density shore zone. They are removed by mass-emergence in early June. Numerically *Pentapedilum uncinatum* is dominant and has a long (9 months) over-wintering generation and a short (1 month), much smaller summer generation.

An estimation of the maximum production (April) for this, the best area in the lake, is 6.3 kg/ha dry weight, other areas are very much lower.

In the sand *Pentapedilum uncinatum* only had one generation per year, but migration from this area of the overwintering 2nd instars provide the spring populations in other favourable areas.

Experiments with fish exclusion cages indicated that for species with higher densities of 4th instars, e.g. *P. uncinatum* 1040/m², suffered a 50% predation from fish, whereas in low density species e.g. *C. anthracinus* 200/m², were inefficiently preyed upon.

It is suggested that a complex of factors cause Chironomid numbers to be low in Tjeukemeer, which prevents fish from extensively using this food source.

Mr. Chambers continued his work on *Chironomus* and *Gammarus* in the dense beds of *Phragmites communis* and *Typha latifolia* that fringe much of the perimeter of the Tjeukemeer and provide a rich source of food for several fish species. The biomass of this food supply shows a marked seasonal variation. It is high in the spring (650 kg/ha) when large numbers of chironomids undergo rapid growth prior to emergence

in May and June. In summer, after chironomid emergence, the fauna has a low biomass (100-150 kg/ha), and this is comprised largely of *Gammarus tigrinus*. Approximately 50 species occur regularly, but no other single species, or group of species (eg. molluscs), make significant contributions to either the numerical or biomass totals.

Gammarus is abundant the whole year round, and most of the total annual production of the *Phragmites*/*Typha* reed bed fauna will be by this one species. Production depends on at least the following factors – numbers, egg and young production, mortality rate, and temperature (growth rate). In the breeding season (April-October), the *Gammarus* population is turned over three times, and from this a minimum estimate of *Gammarus* production can be made. Between May and November, *Gammarus* has a mean weekly production of 17.7 kg/ha, or a total of 478 kg/ha.

Four of the most important fish species of the Tjeukemeer are dependent on the littoral fauna, and principally *Gammarus*, for their food-supply-the eel (*Anguilla anguilla*), pope (*Acerina cernua*), and intermediate stages of the large predatory fish, pike-perch (*Lucioperca lucioperca*), and the perch (*Perca fluviatilis*).

Mr. Goldspink studied the nine common fish species in Tjeukemeer of which three the Pike-perch (*Lucioperca lucioperca*, Roach (*Rutilus rutilus*) and Bream (*Abramis brama*) have been studied in detail. The Pike-perch and eel (*Anguilla anguilla*) form the basis of a thriving fresh water fishery.

In order to estimate the production of these species it was necessary to estimate the number, mortality and growth statistics of the populations. The numbers of roach and bream were estimated using mark and recapture techniques adapted to suit the conditions of the lake. An independent method that involved sub-sampling of the populations using inclosure nets was performed to check the results obtained by the previous methods.

The bream population is not so heavily exploited, such that the biomass consists of many old fish up to an age of fourteen years. Despite this it is a declining population of very slow growth. The production of seven year old fish and above is estimated as 22 kilos/hectare/year of which 3 were removed by the fishery in 1968. They feed mostly upon planktonic crustacea.

The roach is not exploited and is very abundant in the lake, utilising a varied food supply consisting of plants, molluscs and plankton. The production of two year old fish and greater has been estimated at 44 kilos/hectare/year. The growth rate may be said to be average for this type of water, but is not constant from one year to another. The eel in Tjeukemeer is very abundant and can yield up to 6 kilos/hectare/year, most of which is derived from fish, upon which the eel feeds above 28 cms.

The biomass of pike-perch was estimated using the yield statistics of the fishery which is known to remove a substantial amount of the production. The growth and production of pike-perch in Tjeukemeer is good, sufficient to produce a yield of up to 9.5 kilos/hectare/year. The fishery over the years has removed the old fish and most of the catch is derived from fish which are recruited into the fishery at the end of their second year of life. This production is derived from the smelt and the pope upon which the pike-perch feed.

Mr. N. P. van Zalinge measured the growth of the year-classes of pike-perch. It appeared to be about 13.5 cm for the '69 class, 15 cm for the '68 class, 10 cm for the '67 class and 5 cm for the '66 class. Furthermore Mr. van Zalinge made a first attempt to estimate the number of fishes per acre using a net of 600×2.5 m. From fish stomach studies it appeared that smelt (*Osmerus eperlanus* L.) is the most important prey for the pike-perch, although Pope (*Acerina cernua* L.) is important also.

PROJECT IJSELMEER

Drs. de Kloet continued his study of the primary production and the nutrient-balance of the IJselmeer in the North, Middle and South open parts of the lake and in the IJ-meer (A), the Gooi- and Veluwe Border Lakes (R) and in the Ketelmeer (K).

Primary production was measured with the ^{14}C -method in an open tank on board under standardized light conditions. The results are summarized in table 4.

	mg $\text{CO}_2 \cdot \text{m}^{-3} \cdot \text{hr}^{-1}$			Secchi-disk	
	winter	mean	Max. (summer)	mean	range
N)		460	1439	70	(45-130)
M)	25	587	1316	65	(43-127)
S)		632	1343	45	(25- 80)
A	40	540	1422	40	(25- 58)
R	100	1320	7350	45	(25- 83)
K	0	270	806	70	(45- 98)

Table 4. Mean winter- and summer and max. summer values for the $^{14}\text{CO}_2$ uptake and Secchi-disk readings in the different parts of the IJselmeer.

Light penetration was measured with the Secchi-disk and with a Se-fotocell. It appeared that at the Secchi-disk depth (S) the light intensity I_s is about 20% of I_0 , so that

$$\epsilon S = \ln I_0 - \ln I_s = 1.5$$

With the formula $P = F(I) \frac{P_{\max}}{\epsilon}$ and a light factor (energy per day over energy per experimental period) the total photosynthesis may be

calculated assuming that the measured values in the tank equals P_{\max} . Although the latter assumption did not hold true, Drs. de Kloet estimated the total photosynthesis to be 1550-2250 $\text{mg.m}^{-2}.\text{day}^{-1}$ of C per day for the open lake and 1100-4000 $\text{mg.m}^{-2}.\text{day}^{-1}$ for the Border lakes. For the whole IJsselmeer this gives mean estimation of 4000 metric tons of C per day.

Some O_2 determinations were also carried out. It appeared that the gross O_2 -production was in agreement with the CO_2 uptake ($\text{O}_2/\text{CO}_2 = 1.05$); O_2 consumption in the dark was about 10% of the O_2 production. The mean oxygen content of the open area was 95% in the winter and 105-113% during the summer, with peak values of 140% (July) and 170% (September). The concentrations of total phosphate, dissolved phosphates and the sum of ammonia and nitrate nitrogen are summarized in table 5.

	Tot-P		Mean Diss-P in % Tot-P		N mg/l		N/P _{at}	
	mean	range	winter	summer	winter	summer		
N	120	42-259	26	6	2.3	0.93	170	343
M	129	36-294	24	18	2.55	0.70	160	160
S	175	67-347	19	18	2.57	0.91	140	80
A	300	81-704	38	42	2.35	1.55		
R	360	13-1300	33	35				
K	265	148-544	32	33	3.75	3.18		

Table 5. Mean concentrations of total and dissolved phosphate phosphorus and inorganic dissolved nitrogen during summer and winter 1969.

From table 5 and the annual report 1968 it can be seen that the differences of the concentrations in 1968 and 1969 are small.

Phosphate concentrations were estimated in the inflowing waters of the IJsselmeer. From these values and the waterbalance 1962 it is preliminarily estimated that about 12.000 metric tons of phosphate phosphorus enters the IJsselmeer and that only 15% is transported into the Waddensea, so that 10.000 ton remains in the lake.

Drs. Boesewinkel, who studied the phytoplankton found in the open area relatively greater numbers of diatoms (*Asterionella*, *Diatoma*) and *Scenedesmus* in the winter up to and including May, and in the summer *Microcystis aeruginosa*. In the Border Lake R fewer species of algae dominated. In February many diatoms were found (2×10^7 cells/l) and in the summer many *Oscillatoria* and *Aphanizomenon*. In area K blue greens and green algae are dominant in the summer; during the winter very low densities were found.

WIJDE BLIK

Dr. Gulati continued his studies on the Wijde Blik with a one year study on the primary production. For a description of the lake see the

annual report 1968. The lake was sampled at least twice per month in the summer period (except July). Primary production was measured by the C^{14} -method in light and dark bottles in situ. O_2 -concentrations light penetration and temperature were measured in the field at 1 meter intervals from surface to bottom. pH and bicarbonate were measured in the laboratory. The phytoplankton was estimated.

The conversion of 4 hrs primary production values to daily production rates is done with the help of light figures for 24 hrs.

Phytoplankton densities are represented as ind./ml.

Daily solar radiation, average atmospheric temperatures and monthly rainfall figures varied respectively between 130 and 675 Cal/cm², 3°C and 20 °C and between 9.9 and 200 mm during the study.

Light penetration at 1 m decreased from 30% in summer to only 1% in November. The lake had high temperatures in August (26 °C) when it was stratified but otherwise thermal heterogeneity was insignificant – diurnal in nature. Oxygen fluctuations in the lake appeared to be controlled by physical rather than biological processes.

Plankton comprised mainly diatoms and green algae. *Microcystis* was the only dominant blue-green alga assuming dominant proportions in September. There appeared 2 plankton maxima – one in June and the other in October.

The average primary production in the lake was 340 mg C ass/m²/day, the highest being in July (880 mg) and lowest in November (31.2 mg). Dark production averaged 8.4% of the total production. Net production in the lake accounted for 91.6% of the total production of which the upper 5 m contributed to 87.8%.

In general there was a correlation between the primary production, solar radiation, light penetration and temperature in the lake. There was also a broad direct correlation between plankton and production except in July when comparatively low plankton population was accompanied by the highest production for the entire investigation. A second maximum in production during September corresponds with spurt in *Microcystis* in the surface waters.

“VECHTEN” PROJECT

The routine chemical research in lake Vechten was continued again this year. The programme was changed in so far that now mixed samples from different depths are taken.

The work on the mineralization of carbon was re-instigated. For this purpose Miss J. M. C. Jacobs and Mr. T. L. Pons measured the primary production and the increase of total CO₂ in the hypolimnion. When the output of CO₂ of the mud is estimated in the Jenkins mud sampler – which was made in the workshop – it is hoped that the carbon balance can be calculated.

Primary production appeared to be rather constant. The mean value between February and November was 400 ± 100 mg of C per day.

Drs. Boesewinkel studied the periodicity of the phytoplankton and measured the chlorophyll concentration. In the epilimnion this concentration varied in general between 3 and 6 mg/m³, but with a peak of 9 mg/m³ on 6 May. In the hypolimnion a value ten times higher was found at the end of July. There was no indication for a high percentage pheophytin.

The phytoplankton was very poor in 1969. Except for the spring bloom of centric diatoms (ca. 700 cells/ml in April), no algae reached densities higher than 50-100 cells/ml.

Drs. Cappenberg continued his investigations in the possible interaction of sulfate reducing bacteria and methane producing bacteria in their habitat, the mud.

Purified enrichment cultures of methanogenic bacteria were obtained, which fermented formic acid, acetic acid and methanol. With *Desulfovibrio desulfuricans*, a sulfate reducing bacteria, were obtained specific grow rates with different C-sources and electron donors.

Heterotrophic sulfate reducing and methane bacteria were quantitative and qualitative followed throughout the year. The bacterial densities were correlated with the temperature, the pH and the concentrations of oxygen and methane.

The number of heterotrophic bacteria increased during the stratification period up to $2 \cdot 10^8$ bact./l, resulting in an oxygen decrease in the hypolimnion. During the summer a significant peak in the thermocline was found.

Methane oxidizing bacteria are found the whole year. During the stratification period the number increased up to $1.5 \cdot 10^6$ bact./l. In the beginning of the stratification period (May) a maximum was found in the metalimnion and during the stratification period in the hypolimnion, below the peak of the heterotrophic bacteria. The concentration of these bacteria appears to be positively related with the methane concentration and negatively with the oxygen concentration in the water.

The sulfate reducing bacteria were found only two months after the establishment of the hypolimnion. Their number was positively related with the methane concentration and with the concentration of methane oxidizing bacteria.

Drs. Parma continued the sampling of the bottom fauna at four stations. The variation in the density of larvae and pupae of *Chaoborus flavicans* presented the same characteristics as the preceding years. As the genital glands are visible through the cuticle it is possible to determine the sex already in the larval stage. The praepupa stage appeared to be 24-36 hours at 20 °C. Even in mid-winter larvae are to be found in a population of *Chaoborus crystallinus*, which are able to pupate without food. This shows that the population does not need to die out in the absence of food. The

mean life time of unfed larvae that do not pupate is at least 40 days.

From the investigations of Mr. H. F. J. Swüste it appeared that here is a larvae of strong selective predation by *Chaoborus flavicans* on *Diaptomus* in mixtures of *Daphnia pulex* (Cladocera), *Diaptomus gracilis* (Copepoda) and *Cypria ophthalmica* (Ostracoda). *Cypria* is not accepted as food. Mr. R. A. Cremer demonstrated that larvae of *Chaoborus flavicans* may predate on oligochaetes during the benthic phase.

ALGOLOGY

Drs. Steenbergen determined the induction-period for cell division in the lifecycle of *Scenedesmus obliquus* by means of synchronised cultures. The influence of several factors (among others visible light) on this induction-period will be studied. Analytical methods were tested, especially for the chlorophyll determinations in synchronised cultures of *Scenedesmus obliquus*.

H. L. GOLTERMAN

DELTA INSTITUTE FOR HYDROBIOLOGICAL RESEARCH, YERSEKE

PROGRESS REPORT 1969 *

I. INTRODUCTION

The main event of the year 1969 was the start of the rebuilding of the Institute in May and the combined efforts of all concerned to keep „business as usual during rebuilding.” The chemical laboratory was demolished and reestablished at Wemeldinge at a distance of 9 km from Yerseke, in an empty laboratory building, recently evacuated by the Netherlands Institute of Fishery Investigations, owing to its shift to Texel. The aim is to finish the new wing by autumn 1970 and start the rebuilding of the present main building with the intention to end the entire enterprise about March 1971.

The year's progress in the realization of the Delta plan comprised the closure of the Volkerak. As this new dam completely changed the salinity of the entire Volkerak-Haringvliet area, most departments of the Institute were involved in investigations of the changes in the hydro-chemistry of the water and in the composition of the biota. A definite choice was made for the place where the dam across the Oosterschelde is to be constructed. Preliminary constructions on the Roggeplaat have already started.

In the second part of the year all departments collaborated with the Sub-commission SW Netherlands of the Board for Conservancy of Nature, in its efforts to oppose the plans of the City Council of Rotterdam to enlarge their harbours at the cost of the few area's of biological and scenic importance, still left on the Isle of Voorne. Data from previous years, supplemented by others specially gathered for the purpose, were summarized and used to create an ecological evaluation of the area in its present situation and in the projected state of the year 2000, when the Delta plan will be completed and industrialisation and urbanisation will have proceeded to a still further degree. Maps were drawn depicting diversity of benthic algae, fauna of soft and hard substrates along the shores of the water bodies and numbers of higher plants on mud flats and salt marshes, together with salinity, pollution, diversity of plankton and numbers of fishes in those waters. Thus an evaluation was given of the ecological and recreational value of the relevant inland waters. Notes on the avifauna were added.

In the following chapters these activities will be referred to in greater detail.

* Communication no 84 of the Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke, The Netherlands.

II. HYDROGRAPHY AND SOIL STRUCTURE

A. *Hydrochemistry*

Salinity values off Yerseke remained fairly high ($\pm 16^0/_{00}$ Cl') until February, owing to the limited river discharge in winter, and decreased to $14.2^0/_{00}$ Cl' in April. At the end of this month the Volkerak was closed, but, as the actual closure was effected during low tide, the ensuing rise in salinity was rather slow and values about $16^0/_{00}$ Cl' were only reached in September. The monthly measurements of the nitrogen components were continued in the deep pit "Dijkhuis." This year another 20 m deep pit "Caisson" was added to the sampling program. In summer high values for ammonia were found near the completely anoxic bottom, although this pit is not stratified. During the cooler part of the year, when winds could exert more influence, hardly any stratification of the three components nitrate, nitrite and ammonia was found. The investigation of the various compounds of nitrogen was extended to the Grevelingen, the Oosterschelde and to the, 10 m deep pit Schelphoek, created after the flood disaster of 1953, when the new dyke was constructed along the mouth of a newly formed gap. The latter object was chosen in order to obtain an insight into the metabolism of an inland water body of intermediate salinity. As a result of saline seepage from the Oosterschelde the deeper parts show some stratification. Phosphate determination is included in the monthly program.

B. *Measurements of primary production by means of radioactive carbon*

In connection with the current plankton investigations, primary production was measured this year every month in the Veerse Meer (Caisson) and in the Grevelingen. In this work Mr. F. Vegter is greatly hampered by the fact that the countings of the radioactive filtrates still have to be carried out elsewhere.

C. *Hydrology*

Mr. M. C. Daane, soil scientist in Dr. Beeftinks' Department, carried out an investigation on the hydrology and the salinity of the ground-water in the salt marshes, known as Slikken van de Heen, before and after the closure of the Volkerak. As a result of the higher floods and the increase in salinity of the water, the ground-water does not fall as deep as before and its salinity increases in accordance with the salinity of the adjacent open water. Similar relations were observed on other places along the Volkerak.

Four complete annual cycles have been investigated on the salt marshes in front of the Stroodorpolder. The increased influence of the river-water penetrating the Oosterschelde in winter owing to the construction of the Grevelingendam, was clearly demonstrated. Investigations on this and other salt marshes, into the connections between ground-water as well as overflowing seawater and the vegetation, are being continued.

III. ZOOLOGICAL INVESTIGATIONS

A. *Littoral-benthic fauna*

As a result of the closure of the Volkerak, the water of the Haringvliet decreased drastically in salinity and that of the Volkerak increased, which change caused the freshwater molluscs (*Lymnea peregra*) along the Volkerak to disappear suddenly. As a result of the concomittant rise in the waterlevel the numbers of *Assiminea grayana* on the salt marshes along this waterbody decreased and those of *Hydrobia ulvae* increased at some places. Mrs. C. H. Borghouts gave a good deal of attention to the closer study of the *Mysidaceae*. Monthly sampling in the Veerse Meer showed that the numbers obtained during winter decreased rapidly. During the mild weather in summer the opossum shrimps proved to live in the deeper parts during the day and were caught along the shores at night. This migration was studied by simultaneous sampling of the shores, the deeper parts and the surface water over those deeper parts, during the evening and the night. The following, preliminary, conclusion was drawn from the results obtained. The opossum-shrimps – mainly *Neomysis integer* with a few *Praunus flexuosus* – move at night towards the surface and towards the shores. Whether one individual takes part in both movements or not, must be decided by means of marked specimens. These movements are influenced by the light of the sun and the moon. An inventory of *Mysidaceae* in the Delta-area is practically completed. The influence of the dam in the Volkerak was clearly seen in a westward shift in the distribution of *Neomysis integer* in Haringvliet and Hollands Diep.

A canal near Vlissingen forms a natural salt-gradient from a chlorinity of 0.70/00 to 14.0/00 over a distance of 8 km. This canal is sampled every month and *Neomysis integer* proved to be most numerous in two places near either end.

Next to his work on bryozoa, Mr. G. R. Heerebout, a student from Leiden University, wrote an article on an ecological classification of brackish inland waters based on fauna and salinity, with, as regards the latter aspect, the main emphasize on fluctuation. The article will be published soon.

Mr. L. Blommers, a student from Amsterdam University, studied some aspects of the distribution of chironomids in our area. The adults were reared in the laboratory at Amsterdam. He found the genus *Chironomus* to dominate in inland water with a chlorinity exceeding 10/00. A distinct succession was found of species of this genus with decreasing chlorinity: *Ch. salinarius* dominated over 80/00, *Ch. halophilus* usually between 8 and 30/00, the latter two species were also found in practically fresh water.

Mr. A. J. J. Sandee, studying the interstitial microfauna, found that the beaches of Walcheren, where the median grainsize is over 200 μ , are richer than those of Schouwen en Goeree, where lower values were noted.

B. Sublittoral "soft-bottom" fauna

Elaborating the work on polychaetes carried out in previous years, Mr. W. J. Wolff started to compose the first chapters of a treatise on the ecology of the "soft bottom"-fauna of our area, as a base for a doctor's thesis on this subject. Two rare worms were found, viz. *Microphthalmus fragilis* in the mouth of the Westerschelde and *Opisthodonta pterochaeta* in the coastal sea off Schouwen.

The area affected by the closure of the Volkerak was sampled twice. Before the closure a clear-cut gradient could be observed from a rich fauna in the south-western part, under the influence of the Oosterschelde, to a very poor one in the north-eastern part under the influence of the – slightly brackish – main rivers. As a result of the closure the south western part did not change much, but in the other part a distinct spat fall of marine species – cockles and marine worms – was observed in autumn.

Mr. J. G. Koulman, a student from Leiden University, studied the difference between *Cardium edule* and *C. lamarcki*, with morphometric, ecological and cytological methods, in order to decide whether the distinction into two species is valid. His work is nearly finished.

C. Secondary production in the Grevelingen

The biomass of the bottomfauna of the Grevelingen was studied in seven representative associations, by means of the ash-free dry weight of the fauna. From a quantitative point of view the following species are important: *Mytilus edulis*, *Cardium edule*, *Littorina littorea*, *Hydrobia ulvae*, *Arenicola marina* and *Macoma balthica*. For the whole Grevelingen (± 14.000 ha.), the joint ash-free dry weight amounts to 2-3 million kg.

D. Inland waters

Mrs. Borghouts found the fauna of the Veerse Meer changing slowly but perceptibly. In autumn *Hydrobia stagnorum* was found for the first time and later on many were seen along the whole length of the shore. The well known *Dreissena polymorpha* was also found in the lake and in September a wooden raft infected by shipworms was discovered. A survey of the lake established severe infection by *Teredo*'s of wooden structures of various kinds of wood all over the lake. The tube-worm *Mercierella enigmatica* can now be found growing on stones and other hard substrates along the entire shore. The barnacle *Elminius modestus* was found growing sporadically near Kats, but disappeared later on.

Balanus amphitrite amphitrite, found in 1968 in the canal near Vlissingen artificially heated by the effluents of the electricity plant, is now also established in the Kanaal door Walcheren. The question whether the barnacle is growing here permanently or by means of repeated reinfection, is being investigated.

The Brielse Meer, a lake created by construction of a coastal dam before the Delta Plan was born and thus a prototype of the future lakes, was

the object of a study of the bottom fauna carried out by Mr. Wolff. In 30 samples obtained with the Van Veen-grab, he found 3 species of gastropods, a few species of *Pisidium*, many *Dreissena polymorpha*, Oligochetes, *Gammarus duebeni* and insect larvae. On the whole, it was a rather poor fauna, its eastern section still poorer than its western section due to the influence of the Rhine water introduced on the eastern side.

E. Birds

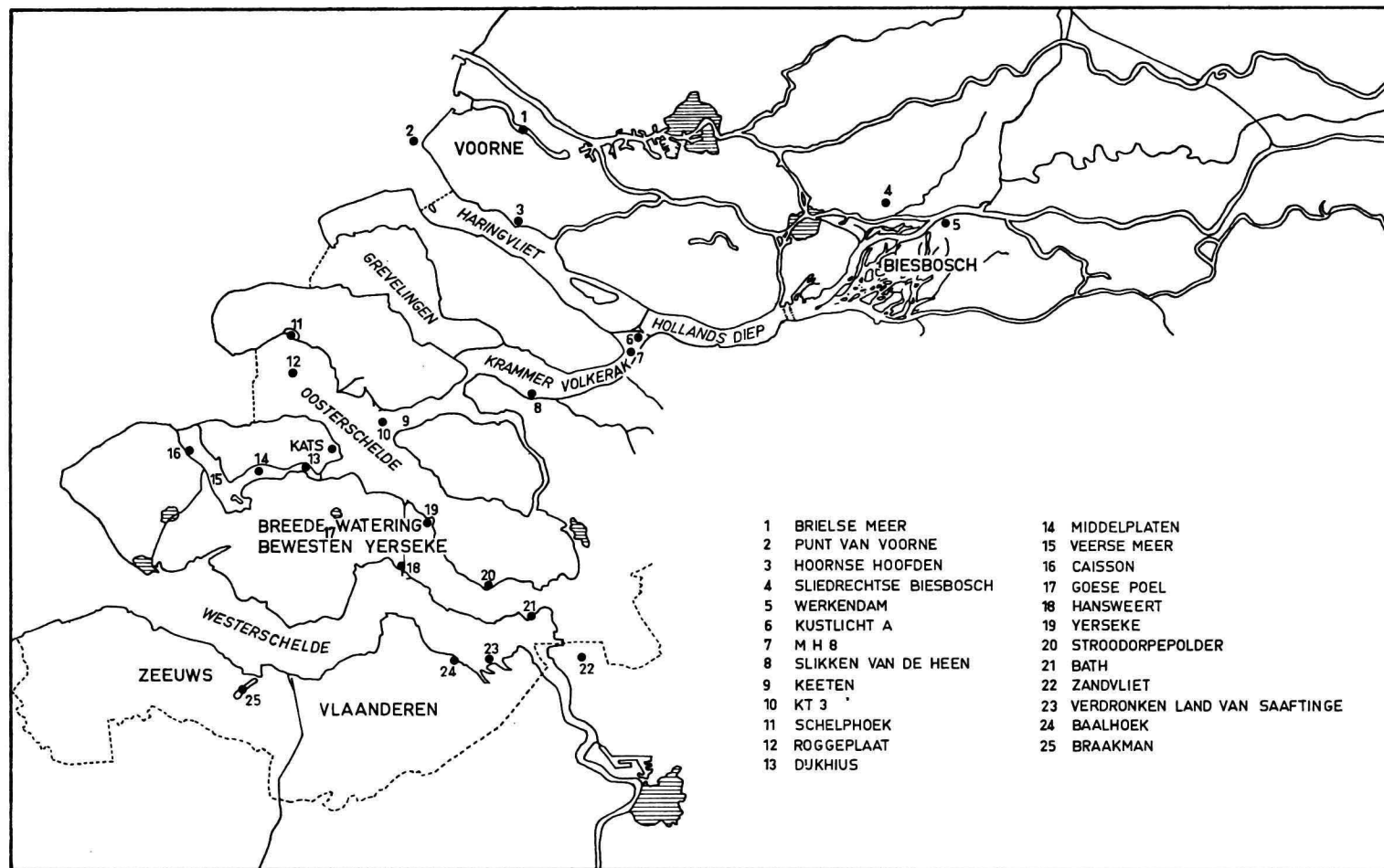
In September the relationship between feeding waders and the bottom fauna of the freshwater tidal area the Biesbosch, was investigated with the collaboration of many amateur bird watchers. The preliminary conclusion was that chironomid-larvae form the principal food of the waders in this biotope.

F. Fish

The manuscript on the changes in the fishfauna of the Veerse Meer induced by the closure and on the results of a closer study of the growth, population dynamics and life cycle of the isolated plaice population, was finished and forwarded for publication. Quantitative fishing was carried out by Dr. K. F. Vaas in the Oosterschelde (5 times), the Westerschelde (once), the Hollands Diep-Haringvliet (twice) and in the Grevelingen (4 times). The results, together with data obtained in previous years, could be summarized in order to create a base for future comparison after the various closures, and, in the case of the Westerschelde, in order to evaluate the progress of pollution. The paucity of marine specimens in the section between Hansweert and the Belgian border, as compared with the western part of the Westerschelde, is to be noted. The Oosterschelde harbours many marine fishes, including some rare ones. The fauna of the Grevelingen is slightly less varied than that of the Oosterschelde and in the Hollands Diep-Haringvliet, at the moment greatly reduced in salinity, the large number of young, migrating flounder and smelt and the enlarged westward extension of the freshwater fishes, were the most outstanding features.

G. Physiological investigations

While investigating the tolerances of *Marinogammarus marinus* and *Orchestia gammarella* against lowered salinities, Dr. A. G. Vlasblom found that, if the females had developed setose oostegites but the juveniles had already left the brood pouch, the tolerance of such females was similar to that of non-ovigerous females and males. So contrary to what is stated in recent literature, the presence of the setae does not always indicate maturity of the ovaries from a physiological point of view. The first set of observations with the microcryoscope on the osmotic pressure of the body fluid of *Marinogammarus* established the fact that,



when the salinity of the environment is lowered to 20‰ Cl', the animal is able to regulate its body fluid hypertonically. Further experiments on the respiration of *Marinogammarus* of different size in combinations of varying salinities and temperatures were carried out. In combinations of 5-10-15 and 20 °C and salinities of 2-4-8 and 16‰ Cl' the respiration of males and females was found to be equal. Oxygen uptake of males over 18 mm in length at 20‰ Cl' is significantly larger than in 4-8 and 16‰, but for males of 20 mm in length the reverse is true. At 15° such differences are not found, and at 10 °C significant differences could be found for all males between 10 and 18 mm in length, with higher uptake of oxygen at the lower salinities. At 16‰ Cl' such differences were only found for the bigger males.

A student of Utrecht University, Miss G. Bolier, studied the tolerance of the eggs of *M. marinus* and *O. gammarella*, using percentage of hatching and rate of development as criteria. The rate of development in 40‰ Cl' proved to be the same as that in 140‰, but the percentage of hatching was lower. Microcryoscopic measurements of the osmotic pressure of the egg-fluid showed that decreases in the environment from 16 to 8 and 4‰ Cl', were closely followed by the eggs.

IV. PLANKTON INVESTIGATIONS

A. Veerse Meer

This year salinity as well as temperature rose to the high values of resp. 110‰ Cl' and 23 °C. *Skeletonema costatum* dominated the plankton in spring and caused supersaturations of oxygen up to 186%, and a drop in Secchi disc-visibility to 50-60 cm. Early in summer the μ -algae developed strongly. Later on during summer, the μ -algae were replaced as dominant plankters by the marine diatom *Coscinodiscus centralis*. Its large cells do not interfere so much with the transmission of sunlight as the minute μ -algae do and therefore visibility increased so as to make the Secchi disc still discernible at the, for Lake Veerse so far, unusual depth of 400 cm. Towards the end of July auxospores of this diatom were observed and a special study was initiated. *C. centralis* was found to grow in two different forms, small cells of 72 μ and large ones of 142 μ . The big ones had just developed from the auxospores and were dividing vigorously, the small ones divided very slowly and gradually disappeared at a length of $\pm 45 \mu$, the critical length where the formation of auxospores is bound to take place in this species. In autumn some other diatoms, previously absent, were seen: *Dytilium brightwelli*, *Biddulphia sinensis*, *Bellerophon malleus*, *Rhizosolenia setigera* and *Nitzschia seriata*.

With the object to obtain a better insight into the oxygen regime of the lake, two periods of 24 hours were investigated. The first one, carried out during the spring outburst of *Skeletonema* was slightly hampered by

a fairly high wind and only limited signs of oxygen stratification could be found. The increase during the day ran parallel to the rise in temperature, from 13.5 to 17 mg/l O₂ with a maximum value about 2 hours before sunset. The supersaturation at the surface decreased gradually during the period. At a depth of 6-7 meters no decrease was found. The lower oxygen values at greater depth remained constant too.

During the second investigation, when wind action was almost nihil, the temperature varied from 20.5 to 22.5 °C and the plankton was only slightly developed. At mid-day the oxygen concentration was about 10 mg/l at the surface and below 2 mg/l near the bottom. During the night the deeper strata remained constant and at the surface the concentration of oxygen decreased to a value of 8 mg/l thus, during the night the upper 3 meters did still show supersaturation and during daytime the point of 100% saturation is situated at a depth of 6 meters.

B. Oosterschelde

In the shallow eastern basin of the Oosterschelde a pit with a depth of 20 m had been dug in order to obtain sand for building purposes. When this work was finished Mr. C. Bakker used this isolated pit for a special investigation. In the 40 m deep gullies in the mouth and central parts of the Oosterschelde no stratification can develop owing to the strong tidal currents. In the shallow basin at the eastern end, those currents are very much weaker and thus some stratification of oxygen was expected in this newly created, deep pit. The assumption proved to be true, as a difference between an oxygen concentration at the surface of 128% and 98% near the bottom was found in spring and an even bigger difference was observed in summer: 102% at the surface and 72% near the bottom. Another interesting find was the presence of large numbers of *Eurytemora affinis* – adults as well as juveniles – in the deeper layers of the pit at the – for this copepod – unusual chlorinity of 14.2‰.

C. Grevelingen

In previous years the development of the plankton in the basin of the Grevelingen, near the dam, had been rather poor, but owing to the fair summer and normal river discharge of this year, about 30 species were observed in countable numbers, more than 3 times the number of 1967.

Dominating were: *Amphora proteus*, *Chaetoceros radians*, *Eucampia zoodiacus* and *Melosira sulcata*, present in numbers varying between 100,000 and 200,000/l. *Asterionella formosa*, derived from the mouth of the Haringvliet, was counted in the seaward part of the Grevelingen, with about 15,000 cells/l. As the surrounding polders did hardly discharge any water, no stratification was found in the deeper parts.

D. Keeten-Krammer-Volkerak

The abrupt changes in salinity in this area, owing to the closure in April, were thoroughly investigated by Mr. R. Peelen. Before the closure

a salt gradient, ranging from a chlorinity, fluctuating annually from 16 to 10‰ Cl' at Kt 3 in the Keeten to 0.3‰ at "Kustlicht A" in Midden Hellegat and with 5 sample places in between with intermediate chlorinities, was repeatedly sampled. *Biddulphia aurita* was found with 3200 cells/l at Kt 3 and showed a gradual decline in numbers, being present with 1000 cells at a chlorinity of about 1‰ at the penultimate sample station MH 8 and totally absent at "Kustlicht A". Both *Skeletonema costatum* and *Streptotheca thamesis* showed a similar distribution. However, the freshwater diatom *Asterionella formosa* was present at "Kustlicht A" with 150,000 cells/l and decreased gradually in a westward direction along the gradient and was absent at salinities over 5.5‰. A totally different situation was found after the closure, although two accidental circumstances had been operating to make the change less abrupt than was expected. Firstly the actual closure took place at the moment of low tide, while the river discharge was twice as large as normally. In the second place a great deal of liquid mud from the Haringvliet was used to close and strengthen the culvert-caissons. Both facts collaborated in slowing down the rate of change from fresh to brackish water. Shortly after the closure, when the chlorinity of the whole tract amounted to, about, 8‰, *Asterionella* was completely absent and *Biddulphia aurita* still showed a gradient in numbers from 12000 cells/l at Kt 3, to zero at "Kustlicht A", but the numbers were smaller than before. Again *Streptotheca thamesis* showed a similar distribution in numbers, but *Skeletonema* demonstrated a totally different pattern. From a value of about 100,000 at Kt 3, numbers were seen to rise gradually and to reach a maximal value of 1,280,000 cells at "Kustlicht A." At this place a "cul de sac" was established and *Skeletonema* seems able to profit from this situation, as does *Eucampia zodiacus*, also found in large numbers here.

E. Hollands Diep – Haringvliet

Due to the closure of the Volkerak, the riverwater forced the seawater still further westwards. *Actinocyclus normannii*, originating in the Biesbosch, extended its area to a marked extent; *Bosmina longirostris* – a freshwater copepod – was found alive near the new bridge over the Haringvliet. The eastern border of the marine plankton is situated near the Hoornse Hoofden during low river discharge. Here we find a mixture of diatoms from the river – e.g. *Tabellaria fenestrata asterionelloides* – and marine diatoms, such as *Triceratium favus* and *Biddulphia rhombus*. Near the "Punt van Voorne", the plankton is entirely marine.

Mr. Peelen gave a good deal of his time during the second part of the year, collaborating with others in- and outside the Institute and coordinating the efforts to react adequately on the harbour extensions plans and other plans put forward by the City Council of Rotterdam. Maps were constructed showing water quality judged by salinity, pollution and plankton, in the present state and about the year 2000.

F. Biesbosch

In this freshwater tidal area the influence of the closure of the Volkerak took the form of a 20% decrease of the tidal action and a decreased turbidity. *Melosira moniliformis*, *Actinocyclus normannii* and some other diatoms predominate in the plankton.

G. Brielse Meer, Brielse Gat and other inland waters

The Brielse Meer once more showed a severe bloom of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. During a study of diurnal variations the oxygen concentration at the surface was found to fluctuate between 120 and 180% saturation, a chemocline was found at a depth of 10-11 metres and under it an oxygen saturation of 3% only was measured.

The Brielse Gat was characterized by a mixture of *Skeletonema costatum* and μ -algae.

An adequate reaction on the plan of the City Council of Rotterdam referred to in earlier chapters, made an ad-hoc evaluation of the many small inland waters in our area inevitable. Mr. Bakker used Heerebout's classification (p. 81) as a base and – with due consideration of the short time available – the results were evaluated as promising. On the whole, those waters proved to harbour a rich and diversified plankton population, where salinity was high as well as constant. Small inland water bodies of an average high or low salinity but characterized by strongly fluctuating salinity, were found to be much poorer in plankton. The inland waters of Zeeuws Vlaanderen – excepting the Braakman and surrounding waters – are characterized by low and fairly constant salinity. They are rich in euryhaline, limnetic species, notably rotifers. However, a few of them are impoverished as a result of pollution.

H. Westerschelde

Mr. N. de Pauw, from the University Centre at Antwerp, Belgium, finished his sampling tours on the Westerschelde this year. For this work, carried out under supervision of Prof. Dr. F. Evens, financial aid is given by the Beyerinck-Popping fund of the Academy. This year tributaries and canals associated with the main river, were studied. They proved to influence the plankton spectre of the Schelde to a marked degree, because their pollution is usually less.

The estuary of the Westerschelde proved to be a "complete mixed estuary", although slight differences between surface and bottom were sometimes observed between Bath and Zandvliet (Belgium). In 1968 the euryhaline diatom *Coscinodiscus biconicus* appeared in the plankton in large numbers during August and September. In previous years *Actinocyclus normannii* was nearly always found in the less brackish part of the estuary with shrunken chloroplasts. This year its original biotope was discovered: the drains of some polders near Uitbergen (Belgium). The horizontal migrations of *Acartia tonsa* and *Eurytemora affinis* were studied

during three tidal cycles of 13 hours each, using three vessels each time. The fact that the latter copepod is most frequent in the area between Baalhoek and Zandvliet, seems to be explained by the hydrographical structure of the estuary in the region of the Verdrongen Land van Saaftinge.

V. BOTANICAL INVESTIGATIONS

A. *Veerse Meer*

This year Dr. W. G. Beeftink, continuing his observations on the vegetation of the Middelplaten, observed a continual increase in the development of the grass vegetation. The orchid, first seen in 1968, was identified as *Orchis praetermissa*.

B. *Halophytic vegetation elsewhere in the Delta Area*

The abrupt increase in tidal fluctuation along the Volkerak, as well as the gradual rise in salinity induced a scala of reactions from the phanerogams growing along the shores. The following reactions could be studied. Burning and withering, as demonstrated by the *Agropyro-Rumicion*-associations along the Zijpe, local die off – *Halimione portulacoides*, *Festuca rubra* and *Elytrigia pungens* on the Slikken van de Heen-absence of flowering – *Juncus gerardii*-, and some others. *Aster tripolium* and *Salicornia europaea* did not show any reactions so far and *Triglochin maritima*, as well as *Spartina townsendii*, reacted positively with better growth. Along the Haringvliet no reaction of the higher plants could be found as yet.

The inventory of shore vegetation and plants growing on the outside slopes of dykes, was continued this year, mainly in the north-eastern part of the region. As a result of this work the distribution pattern of about 500 species could be ascertained and classified. Special attention was paid to the vegetation of river-dunes in the Sliedrechtse Biesbosch and the area upstream of Werkendam. These dry, calcarious deposits are the habitat of the westernmost offshoots of the subatlantic and continental grass-land communities, confined to river-valleys in the Netherlands.

Prof. Dr. J. L. van Soest (Leiden) distinguished 57 taxa in the material of the genus *Taraxacum*, collected so far. Sociological surveys were made of associations including *Taraxacum limnanthes* ssp. *limanthoides*, the taxon most tolerant to salinity.

Several reports on the work of students, carried out last years, were finished. In his study on the variability of *Aster tripolium*, D. O. Wijnands (Amsterdam) came to the conclusion that the var. *solstitialis* Focke, as well as the var. *autumnale* Focke are no valid taxa in our area, as their differential characteristics were found to be more closely correlated with certain environmental features than with one another. The f. *discooides* had greatly extended its territory since, about 1880 and is still extending. The genus is potentially perennial, but the environment decides, whether

perennial growth will be realized. Five forms could be distinguished in the Netherlands.

C. van der Kraan (Free University, Amsterdam) established various statistically significant differences in the presence of a number of species growing on outer dykes as regards grazing by cattle and sheep, exposition and position on the inside or outside of the dyke.

The report of G. C. Redeker (Utrecht) described the vegetation of cattle-pools in the Goesse Poel, consisting of 7 communities of aquatic plants and 10 associations of plants growing along the borders. The depth of the pools and their salinity proved to be the principal environmental factors as far as the vegetation is concerned, with slope, shading and distance between edge and waterlevel as factors of secondary importance.

J. C. H. Peeters (Leiden) studied the hedges in the eastern part of the polders "De Breede Watering bewesten Yerseke." The association *Solano-Crataegetum*, to which these hedges belong, consists of two variants: one characterized by *Bryonia dioica*, the other by *Geum urbanum* and *Geranium robertianum*. Two marginal types were distinguished: a nitrophilous type with species from the class *Artemisietea* and a second type forming a transition towards the alliance *Trifolion medii*.

C. Benthic Algae

Studying the results of his algal work carried out so far, Mr. P. H. Nienhuis came to the conclusion that epilithic algae, as well as algae from soft substrates growing along the coasts and along the borders of inland waters, might well be studied from the same point of view, as they all are living in the contact zone between the aquatic and terrestrial environment. This biotope is characterized by its extreme fluctuations in chemical and physical water-regime. The many algal species growing there all show adaptations for this extreme environment in their morphology, ecology and reproduction. For this reason characteristic representatives, such as *Rhizoclonium riparium* and the genera *Enteromorpha*, *Blidingia* and *Microcoleus*, received special attention and efforts were made to correlate changes in the vegetation of these algae with changes in the environment.

Along the Volkerak no new species were observed, in spite of the suddenly increased salinity of the water and the soil. Loose-lying species were very often swept away or covered with sediments as a result of the higher water level. On the slopes of the dykes the algal vegetation was found to grow some decimetres higher.

As practically all red-algae are bound to disappear after the complete realization of the Delta Plan, this group is continuously studied and some species, new for the Dutch flora, were found this year: *Rhodochorton floridulum* (Dillw.) Näg. in oysterpits at Yerseke and *Gelidium pusillum* (Stackh.) Le Jol. along the Grevelingen near Ouddorp.

Along with many disappointments in his comprehensive work on

culturing of algae in vitro, Mr. Nienhuis scored the success of observing several times the sporulation, the hatching of zoöspores and the fusion of the gametes of *Rhizoclonium* cultures.

K. F. VAAS.

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74. WOLFF, W. J.: A new station of *Ophelia rathkei* McIntosh in northern France. *Cah. de Biol. Mar.* 10, 83-84 (1969).
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