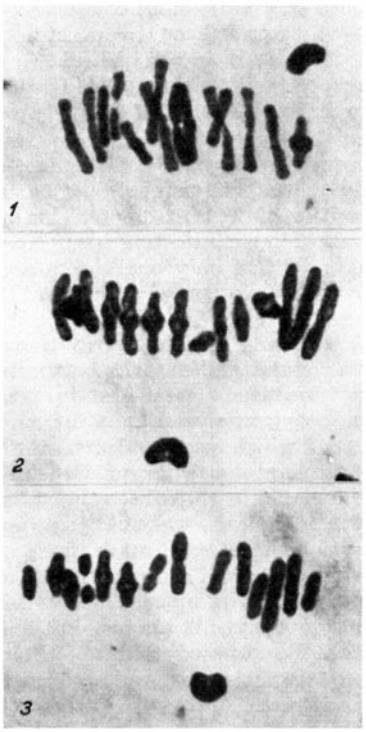
An attempt was made to elucidate the probable adaptive value of the different chromosomal types by investigating seasonal changes in their frequencies. This analysis, which is being continued, has so far revealed significant differences between the frequencies of the karyotypes in spring and autumn populations, respectively.



Figures 1–3.–*Ameles heldreichi*, first metaphases of meiosis. Aceto-orcein squashes. × 1900.

Fig. 1.–Type A, one ring-shaped and 12 rod-shaped bivalents.
Fig. 2.–Type B, one trivalent and 12 rod-shaped bivalents.
Fig. 3.–Type C, 14 rod-shaped bivalents.

The Amelinae thus afford a striking instance of a variation in chromosome numbers according to the so-called ROBERTSON's "Law", the number of chromosome arms remaining constant. It seems plausible that the process is actively going on at present since its intermediate stages are preserved intra-specifically, probably owing to differences in the adaptive values of the types involved.

It must be expected that active chromosome evolution, conforming to ROBERTSON's principle, should produce structural heterozygotes of this type, but these were rarely observed. This is the first record for Mantids but similar conditions were reported for a few natural populations of other Orthopterans. Unfortunately most published accounts are based on rather scarce material. A small population of *Hesperotettix viridis*, variable in chromosome numbers, was found by MCCLUNG[1] to include several individuals with trivalents. In *Loxoblemmus arietulus* an intraspecific variation was described[2] and later confirmed[3]. In this case, too, the even chromosome number is maintained by a trivalent. An unpublished thesis[1], mentioned in a recent review on the cytogenetics of the Orthopteroid insects[2], deals with a particular population of *Circotettix undulatus* the members of which also show this kind of polymorphism; the same being true for *Trimerotropis sparsa*[3]. Among ten individuals of three species of the genus *Jamaicana*, which are related according to ROBERTSON's "Law", a single heterozygous one was found[4]. Outside the Orthoptera the Anguiid lizards of the genus *Gerrhonotus* should be mentioned[5]. Although in this instance no meiotic stages with a trivalent were observed, the chromosomal variation within the species investigated suggests that a similar polymorphism might exist.

As regards the group of Mantids the importance of ROBERTSON's rule in their chromosomal changes is indicated by the existence of two additional instances of the same type of intra-specific polymophism as described above. This polymorphism was found in another, yet undetermined, species of *Ameles* and in *Iris oratoria* (author, unpublished).

A full account of this work will be published elsewhere.

I wish to express my gratitude to Dr. E. GOLDSCHMIDT for her interest and helpful criticism. Several taxonomic problems were discussed with Dr. B. P. UVAROV, F. R. S., to whom my sincere thanks are due.

J. WAHRMAN

Department of Zoology, The Hebrew University, Jerusalem, Israel, October 9, 1953.

Résumé

Observations sur la variation intra-spécifique du nombre des chromosomes dans deux espèces du genre *Ameles*. On décrit ici 3 types chromosomiaux apparaissant chez *A. heldreichi*. Les chromosomes de ces 3 types, de même que ceux de 4 espèces apparentées à *A. heldreichi* ont d'une façon constante 30 bras. L'évolution chromosomique s'effectue selon le principe de ROBERTSON et est probablement très active puisque on trouve des états intermédiaires: des individus hétérozygotes pour un élément métacentrique et deux éléments acrocentriques. Les 3 types cytologiques décrits par nous en Israël se retrouvent dans une population d'*A. heldreichi* provenant de la Turquie.

[1] C. E. MCCLUNG, J. Morph. *29*, 519 (1917).
[2] H. HONDA, Proc. Imp. Acad., Japan *2*, 562 (1926).
[3] F. OHMACHI, Stud. Tokugawa Inst. Tokyo *3*, 1 (1935). – K. SUZUKI, Proc. Imp. Acad. Japan *9*, 330 (1933).
[1] W. L. EVANS, Unpublished thesis, Univ. of Texas, 1950.
[2] M. J. D. WHITE, Advances in Genetics *4*, 267 (1951).
[3] M. J. D. WHITE, Evol. *5*, 376 (1951).
[4] C. I. WOOLSEY, Biol. Bull. *28*, 163 (1915).
[5] R. MATTHEY, *Les Chromosomes des Vertébrés* (Rouge, Lausanne 1949).

Is there any Quantitative Relationship Between the Synthesis and the Breakdown of Nucleic Acids in Living Cells?[1]

In a recent paper, STEVENS, DAOUST, and LEBLOND[2] advocated, on the basis of their tracer experiments, the

[1] Supported in part by the Scientific Research Fund of the Ministry of Education given to the Synthetic Research Group on "Nucleic Acids". The warm encouragement given to this work by Prof. J. KAMAHORA of this Institute is heartily acknowledged. We are further indebted to Dr. T. MIURA, Department of Radiology, University of Osaka Medical School, for his help in this work.
[2] C. E. STEVENS, R. DAOUST, and C. P. LEBLOND, J. biol. Chem. *202*, 177 (1953).

view expressed originally by HEVESY that the[1] mitosis involves synthesis of two new DNA molecules accompanied by a breakdown of one old DNA molecule, resulting in a doubling of DNA content of the dividing cell. The same idea had been presented by SIBATANI[2] as a hypothesis on the mechanism of self-duplication in relation to the biosynthesis of PNA (pentose nucleic acid) as well as DNA (desoxyribose nucleic acid). He had speculated that Nature made possible the maintenance of the macromolecular specificity of nucleic acids throughout successive generations of self-propagating systems only through a physical discontinuity of individual specificity-carrying molecules pertaining to each generation. At that time data of STANLEY[3] and of PUTNAM and KOZLOFF[4] indicating an extensive breakdown of nucleic acids of infecting viruses seemed to support this hypothesis. It should be mentioned here that SIMPSON[5] has recently suggested that the biosynthesis of proteins in surviving tissues may be connected with the release of amino acids from preformed protein molecules.

But such an idea is apparently incompatible with the fact that in growing bacteria the turnover of nucleic acids and protein is not so extensive as is predicted in that theory, and may be even negligible[6]. KOZLOFF[7] was inclined to accept the nonspecific transfer of parental phage DNA to progeny which may imply the breakdown of DNA molecules to non-specific fragments. According to WATSON and MAALØE[8], however, there is no evidence which excludes the transfer of intact DNA molecules from parent to progeny phages.

It thus seems that the hypothesis of HEVESY, SIBATANI, and STEVENS *et al.* is of no general significance for the mechanism of DNA biosynthesis. Besides, there is a certain weak point in the experiments of STEVENS *et al.* They estimated the rate of mitosis but not the rate of actual increment of DNA in a given system. In order to explore the same problem, we conducted some preliminary experiments with bacteria and rat liver.

Experiments were designed to estimate the increase in total amount and the decrease in total radioactivity of DNA and PNA of P^{32}-labelled growing systems[9]. This kind of experiment is profitable because the possibility of contamination of nucleic acid fractions with compounds of high specific activity[10] can be ruled out with such materials. Nevertheless, combined nucleic acids were first isolated and purified, and then divided into DNA and PNA, essentially according to Barnum *et al.*[11]

When necessary, protein was removed from final solutions with chloroform. Inorganic phosphate was separated by LEPAGE's method[1]. Aliquots of the test materials were used for determination of nucleic acids by the SCHNEIDER method[2]; DNA-P and PNA-P in the final samples for radioactivity measurements were determined by the usual sugar reactions[3] or phosphorus determination by modified GOMORI method[4]. The two methods of determination, when employed together, gave satisfactory agreements. Again, two separately purified specimens of DNA from one and the same liver homogenate gave almost the same specific activity values.

Table I.—Turnover of DNA and PNA phosphorus in growing *Escherichia coli*

Time h	μg Phosphorus in ml culture		Spec. activity count/min/μg P		Total activity count/min/ml culture	
	DNA	PNA	DNA	PNA	DNA	PNA
0	2·97	9·50	25·0	23·8	74·2	226
1	3·99	16·5	17·3	13·5	68·9	223
2	5·04	34·4	15·2	8·68	76·6	298

P^{32}-labelled cells of *Escherichia coli* B[5] were obtained by inoculating in a lactate medium containing 0·2 per cent casein acid-hydrolysate and $2·3 \times 10^5$ counts/min of P^{32}-orthophosphate. After growing for 17 h (6×10^9 cells/ml), the bacteria were harvested, washed twice with 0·03 M phosphate buffer of pH 7·0 and suspended in 250 ml of new unlabelled medium of the same composition as used before, and allowed to proliferate by shaking at 37°. The quantity of DNA-P and PNA-P per ml of the culture was measured at 0, 1, and 2 h of incubation, and correlated with the change in total activity of DNA and PNA per ml culture. The secondary incorporation of P^{32} from bacterial acid-soluble materials was neglected. Results of a typical experiment is shown in Table I. It is seen that there is no extensive (if any) breakdown of esther DNA or PNA during the growth for 2 h, while DNA and PNA showed 1·7- and 3·6-fold increase, respectively. The data are in line with those mentioned by PUTNAM[6], and those of MANSON[7] using C^{14}-glycine with *E. coli* B. Results of similar experiments by SCHADE *et al.*[8] on the normal growth of *Proteus vulgaris* showed slight but consistent breakdown of both nucleic acids in the majority of cases, but the specific activities of DNA and PNA of initial labelled bacteria, as calculated from their data, do not agree very well, suggesting involvement of appreciable errors in the measurement.

Another experiment was carried out with a litter of young growing rats. A lactating mother rat weighing about 200 g was injected 0·1 mc of P^{32}-orthophosphate per day for 10 days from the day after parturition.

[1] G. HEVESY, Adv. biol. med. Physics *1*, 409 (1948).

[2] A. SIBATANI, *Nucleic acids and nucleoproteins* (edit. by F. Egami, Tokyo), *2*, 142, 186 (1951), in Japanese.

[3] W. M. STANLEY, J. gen. Physiol. *25*, 881 (1942).

[4] F. W. PUTNAM and L. M. KOZLOFF, J. biol. Chem. *182*, 243 (1950).

[5] M. V. SIMPSON, J. biol. Chem. *201*, 143 (1953).

[6] H. B. LEVY, E. T. SKUTCH, and A. L. SCHADE, Arch. Biochem. *24*, 206 (1949). – F. W. PUTNAM, Expt. Cell Res. Supplem. *2*, 346 (1952).

[7] L. M. KOZLOFF, J. biol. Chem. *194*, 95 (1952).

[8] J. D. WATSON and O. MAALØE, Biochim. Biophys. Acta *10*, 432 (1953).

[9] The P^{32} used was supplied by the U. S. Atomic Energy Commission, Oak Ridge, Tennessee. We express our gratitude to Prof. I. HONJO and his associates, Department of Biology, University of Osaka, for the facilities in using the end-window type GEIGER-MÜLLER counter and advise and help in the measurement of radioactivity.

[10] J. N. DAVIDSON, S. C. FRAZER, and W. C. HUTCHISON, Biochem. J. *49*, 311 (1951).

[11] C. P. BARNUM, C. W. NASH, E. JENNINGS, O. NYGAARD, and H. VERMUND, Arch. Biochem. *25*, 376 (1950). – C. P. BARNUM and R. A. HUSEBY, Arch. Biochem. *29*, 7 (1950).

[1] G. A. LEPAGE, *Manometric techniques and tissue metabolism* (Ed. by W. W. UMBREIT *et al.*, Minneapolis, 1949), p. 209.

[2] W. C. SCHNEIDER, J. biol. Chem. *161*, 293 (1945).

[3] Y. YAGI, *Nucleic acids and nucleoproteins* (edit. by F. Egami, Tokyo) *1*, 132 (1951), in Japanese.

[4] G. GOMORI, J. Lab. Cli. Med. *27*, 955 (1942).

[5] The original strain was received from Prof. N. HIGASHI, Department of Microbiology, University of Kyoto Medical School.

[6] F. W. PUTNAM, Expt. Cell Res. Supplem. *2*, 346 (1952).

[7] L. A. MANSON, Fed. Proc. *12*, 242 (1953).

[8] H. B. LEVY, E. T. SKUTCH, and A. L. SCHADE, Arch. Biochem. *24*, 206 (1949).

Table II.—Turnover of DNA and PNA phosphorus in growing young rat

Age day	mg Phosphorus per liver		Specific activity count/min/mg P			Total activity count/min/liver		Corrected total activity count/min/liver	
	DNA	PNA	DNA	PNA	inorg. P.	DNA	PNA	DNA	PNA
20	0·291	0·908	6390	5630	3040	1860	5180	1860	5180
30	0·517	2·52	3410	1820	423	1760	4580	1370	1790

Having administered total of 1 mc P^{32}, suckling babies were separated from the mother and delivered to another lactating female rat which had not received P^{32}, in order that the compounds with high specific activity in sucklings should be turned over. The growth of the babies was normal. After ten days of lactation by the foster-rat, 6 radioactive weanlings were obtained. They were then divided into 4 and 2 rats, the ratio of the total body weights of the two groups being just 2:1. The former group was sacrificed directly, the latter group after being allowed to grow for another ten day period. DNA and PNA were purified from pooled livers in each case. The total activity of two nucleic acids per liver did not decrease significantly during the 10 day period of rapid growth of the liver, which corresponded to body weights from 28 to 53 g[1] (Table II). But, considering the pretty high specific activity of inorganic phosphate in the liver of younger rats, correction for the secondary incorporation was made by assuming daily linear increase of PNA and DNA per liver and daily exponential decrease in specific activity of inorganic phosphate as the precursor of nucleic acid-P. The corrected total activity[2] now reveals that there was very probably a breakdown of PNA, and to a lesser extent, of DNA, too, during the growth of the liver, but the extent of this breakdown cannot be correlated in any simple way with the actual increment of PNA and DNA per liver.

These results would, then, suggest that the mechanism of nucleic acid biosynthesis does not necessarily involve a breakdown of the pre-existent molecules of the same compound.

Y. FUJISAWA and A. SIBATANI[3]

Microbial Diseases Research Institute, University of Osaka, Doozima-Nisimati 7, Kitaku, Osaka, Japan, August 22, 1953.

Zusammenfassung

Es wurde untersucht, ob die Biosynthese der Nukleinsäuren in wachsenden Systemen mit dem Abbau von ursprünglich vorhandenen Nukleinsäuren derselben Art gekoppelt ist. Die Versuche wurden mit Bakterien (*E. coli*) und mit Rattenleber gemacht. Die in der Zelle vorhandenen Nukleinsäuren waren mit P^{32} markiert, und es wurde der Verlust an Radioaktivität in jeder der beiden Nukleinsäuretypen im Laufe des Wachstums gemessen. Die Resultate wiesen darauf hin, dass das Auftreten von neuen Nukleinsäuremolekeln in der lebenden Zelle ohne den begleitenden Abbau der alten Molekeln stattfinden kann.

[1] M. FUKUDA and A. SIBATANI, J. Biochem. (Japan) *40*, 95 (1953).

[2] The corrected values may be a little too low, because the estimated activity increment due to the secondary incorporation of P^{32} into nucleic acids was subtracted from the observed total activity of the respective nucleic acids obtained from the 30 day old rats, neglecting the loss of activity due to the possible breakdown of nucleic acid molecules containing secondarily incorporated P^{32}.

[3] *Present address:* Department of Cytochemistry and Histology, Yamaguti Medical School, Nakaube, Ube, Yamaguti-Ken, Japan.

Temperature-Dependent Cellulase Production by *Neurospora crassa* and Its Ecological Implications

The ascomycete *Neurospora crassa* SHEAR and DODGE has in recent years become an important tool in biochemical genetics. The mold is usually grown on liquid or agar-solidified synthetic media containing inorganic salts, biotin, and a suitable carbon source. In the course of some recent experiments it became necessary to modify this procedure slightly by growing the mold in a glycerol-containing liquid minimal medium on filterpaper. Under these conditions the mold produces a considerable amount of glucose at 35° C but only a very small amount at 25° C. The sugar is produced only in the presence of filterpaper and its formation is due to the production of an extracellular cellulase.

Neurospora crassa strain W 2/49 A is used; for one of the experiments, strains Abbott a and Chilton a are also used. The medium employed is a WESTERGAARD and MITCHELL[1] medium containing as the carbon source 20 ml of glycerol per litre; 15 ml are used per PETRI dish. To each dish are added 5 sheets of sterile WHATMAN No. 4 filterpaper, 8 cm in diameter. The uninoculated, autoclaved medium gives negative BENEDICT and MOLISCH tests. The filterpaper also is free of reducing substances as well as soluble starch. Inoculation is made by adding to each dish 0·5 ml of a suspension of conidia in sterile distilled water. The temperature of incubation is 35° C or 25° C.

When the organism is incubated at 35°C, a considerable amount of a reducing sugar accumulates in the medium. This sugar gives positive BENEDICT and MOLISCH reactions, and negative BARFOED, SELIVANOFF, mucic acid, phloroglucinol-HCl, and benzidine tests. Microscopic examination of the osazone shows a typical glucosazone. An elementary analysis of this osazone gives the following results:

glucosazone	calculated	C 60·32 H 6·19 N 15·63,
$C_{18}H_{22}O_4N_4$	found	C 60·18 H 6·34 N 15·68.

Final identification of the sugar is made by paper chromatography, using WHATMAN No. 1 filterpaper, butanol-acetic acid-water (4:1:5, v/v) as the solvent, and aniline hydrogen phthalate as the developing agent. The unknown sugar moves at the same speed as glucose, and gives only one spot when mixed with a sample of authentic glucose. The amount of glucose found varies due to its continual utilization by the organism, but it was calculated that in some of the experiments roughly

[1] M. WESTERGAARD and H. K. MITCHELL, Amer. J. Botany *34*, 573 (1947).