Formation of Histamine in Transplants from a Rat Mammary Carcinoma

A high rate of histamine formation has been recognized as part of various types of normal rapid tissue growth[1]. The rat embryo, during a certain phase of growth, exhibits a high histamine forming capacity (HFC) as a result of activation of histidine decarboxylase[2,3]. Embryonic growth can be arrested by enzyme inhibition[4]. In reparatively growing tissues of healing skin wounds, the HFC was 50–60 times the level in control skin[5]. The rate of wound healing in rats, as measured by the tensile strength of the wound, could be retarded or enhanced by measures which depressed or elevated the HFC[5]. The rate of collagen formation in healing skin wounds could be increased by elevating the HFC[6]. Extracellular histamine derived from injected 'long-acting-histamine' (histamine dipicrate) had no effect on the rate of healing or collagen formation[6]. The histamine content of these tissues with a high HFC was very low, and mast cells were largely absent. In man the histidine decarboxylase activity is higher in blood containing immature myeloic cells, capable of mitosis, than in blood where these cells are few or absent[7].

Once the relationship between histidine decarboxylase and normal rapid tissue growth was recognized, it appeared likely that a similar association would be found also in malignant growths. Accordingly, in rats bearing a subcutaneously implanted hepatoma, a high rate of histamine formation was found which fell to normal on removal of the tumour; the histidine-decarboxylase activity was high in homogenates of hepatoma tissue and low in normal rat liver[8].

The object of the present study was to determine the HFC of a rat cancer tissue *in vitro* by a sensitive method permitting the use of low, physiological concentrations of histidine. The tumour tissue was obtained from female albino rats bearing subcutaneous transplants of the Walker rat mammary carcinoma 256. Portions of 1 g of minced cancer tissue. after suspension in 3 ml of 0.1 *M* phosphate buffer of pH 7.3, were incubated with 40 µg ^{14}C-histidine for 3 h at 37°C under nitrogen. The amount of ^{14}C-histamine formed during the incubation was determined by isotope dilution with histamine dihydrochloride as carrier according to SCHAYER, by the standard procedures as described in detail[9]. The cancer tissue produced histamine at considerable rates. In 4 determinations on 4 separate tumours respectively 0.094, 0.096, 0.139, and 0.154 µg ^{14}C-histamine (in terms of the base) was formed per g tissue in 3 h. The histidine decarboxylase activity of the tumour tissue was considerably increased by the addition of pyridoxal-5-phosphate. In normal rat mammary tissue, excised from pregnant rats 20–21 days after mating, the rate of histamine formation was too low to permit exact determinations. The histamine content of the malignant and normal mammary tissue was very low, in the range of 2 µg/g (base). Mastcells were not found in the tumour tissue investigated. A similar discrepancy between high HFC and low histamine content is characteristic of embryonic and granulation tissue where the high HFC is non-mast cell in nature.

The present observations are in line with reports from this institute which suggest that rapidly formed intracellular histamine actively and locally promotes certain types of tissue growth.

Zusammenfassung. Tumorgewebe von Ratten mit subcutan transplantiertem Walker-Milchdrüsencarcinom 256 produzierte ^{14}C-Histamin mit beträchtlicher Geschwindigkeit bei Inkubation des Krebsgewebes mit ^{14}C-Histidin. Zusatz von Pyridoxal-5-phosphat aktivierte die Histidindecarboxylase des Krebsgewebes. In normalem Milchdrüsengewebe lag die Histaminbildung ausserhalb des Messbereichs. Der Histamingehalt des Krebsgewebes war sehr niedrig und Mastzellen wurden keine gefunden. Der Histaminmetabolismus des Carcinoms 256 ist demjenigen im Rattenembryo und im Granulationsgewebe heilender Hautwunden ähnlich.

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In vitro Uncoupling of Oxidative Phosphorylation in Normal Liver Mitochondria by Serum of Sarcoma-Bearing Rats

In previous reports (NANNI[1]), an uncoupling of oxidative phosphorylation was observed in liver and kidney mitochondria of sarcoma-bearing rats. Treatment *in vivo* with, and addition *in vitro* of, extracts of cortical or of necrotic central parts of this tumour cause in liver mitochondria of normal animals an evident uncoupling of phosphorylations from oxidations, greater in the first case than in the second. In the case of kidney mitochondria, treatment with the two kinds of extracts does not modify oxidative phosphorylation. It would therefore seem that a substance (or several substances) forming the soluble phase of cortical zone neoplastic cells is able to act on mitochondria containing enzymes of oxidative phosphorylation, producing an uncoupling of phosphorylation from oxidation. Work by many authors has demonstrated that neoplastic tissues give out substances which pass into the circulation (SHERMAN et al.[2]); FUKUOKA and NAKAHARA[3] have shown a thermostable protein in the serum of sarcoma-bearing animals. Other authors demonstrated inhibitive actions on many enzymatic activities by tumour-elaborated substances (ELLIOT[4], DIANZANI[5], EMMELOT and

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Bos[6]); Rose[7] observed that the serum of sarcoma-bearing animals has a picnotic activity on HeLa cells. In the serum a hypoalbuminaemia was found in tumour-bearing animals (Mider et al.[8]), with an increase of α_2- and β-globulins (Clausen et al.[9]). Many investigators observed an increase in plasma mucoproteins belonging to the α_2-globulins (Seibert et al.[10]). Miller and Bernfeld[11] demonstrated an anomalous protein in the plasma of C_3H mice with spontaneous sarcoma. The present investigation was undertaken to ascertain whether the substance (or substances) responsible for the uncoupling of oxidative phosphorylation in Galliera-sarcoma-bearing rats was present also in their serum.

This investigation was made on Galliera sarcoma-bearing albino rats. This is a spontaneous tumour of rats, isolated in 1920 and preserved by regular transplantation. The sarcoma appears to consist of a growing cortical zone and of a central zone, in which necrosis phenomena are predominant. Microscopically, it appears to be a polymorpha cell sarcoma (Novelli[12]). For the preparation of serum we used normal rats and tumour-bearing rats, 25 days after grafting. The animals were bled to death and the blood was collected in sterile test tubes, the serum was used within 24 h. Liver mitochondria were isolated, as previously described (Nanni[1]), from normal albino rats, weighing 150–170 g, fed on a standard diet. Oxidative phosphorylation was determined using 0.01 *M* K-glutamate as substrate. O_2 uptake was measured manometrically, using Warburg flasks equipped with one side-arm, with air as gas phase, at 25°C. Inorganic orthophosphate phosphorus was determined by the method of Fiske and Subbarow[13]. An amount of liver mitochondria corresponding to 200 mg of fresh tissue (about 1.5 mg N) per Warburg flask, was used. When serum activity was tested, 0.2 ml normal serum, as control, or 0.2 ml serum from sarcoma-bearing rats, were added to each flask. ATPase activity was determined in normal serum and also in serum of tumour-bearing animals, according to the method of Dubois and Potter[14]. Nitrogen estimations were made by the usual microKjeldhal technique. The standard deviation and Student's 't' test being calculated for each average.

Table I shows the data concerning the behaviour of oxidative phosphorylation in three different series of experiments: in normal liver mitochondria, the effect of the addition *in vitro* of normal rat serum or of serum from sarcoma-bearing rats to normal liver mitochondria. In the first two series of experiments, made as controls, oxidative phosphorylation was found to be normal. In investigations made with addition of sarcoma-bearing rat serum, there is an evident uncoupling of phosphorylations from oxidations in normal liver mitochondria. Also in this case, as in previous investigations (Nanni[1]), the decrease in phosphorylations is accompanied by a decrease in oxidations; this decrease does not run parallel to that of phosphorylations, therefore the P:O ratios are impaired. Among the possible reasons for this behaviour, the presence in the serum of tumour-bearing rats of a type of ATPase activity, which would destroy ATP as soon as it is formed, has been considered. A decrease of ATPase has been reported from many authors (Allard et al.[15]) in hepatomas; according to others, on the contrary, it is normal (Potter and Liebl[16]). Serum from sarcoma-bearing rats was therefore examined: ATPase activity was found to be very slight, lower even than the minimum activity which is found in normal serum (Table II). It is therefore possible that serum from sarcoma-bearing rats contains a substance or several substances which are able to produce an uncoupling of oxidative phosphorylation in animal organs. This uncoupling agent, according to previous reports, might derive from neoplastic tissue metabolism and would have the same effect *in vivo* and *in vitro*. The observed uncoupling seems to be due to a greater decrease of phosphorylations than of oxidations, with a consequent alteration of P:O ratios. It may be thought that neoplastic cell components, through the blood circulation, act on mitochondrial membrane permeability, thereby damaging the normal action of mitochondrial enzymes in the various organs of tumour-bearing animals. We cannot at present specify the mode of action of neoplastic tissue on oxidative phosphorylation; it is possible that it acts either by releasing mitochrome from mitochondria (Pullman et al.[17], Polis et al.[18], Sacktor et al.[19], Lehninger et al.[20], Laudahn[21]), or in some other way. Further work on these problems is in progress.

Tab. I. Influence of addition *in vitro* of normal rat serum or of serum from sarcoma-bearing rats on oxidative phosphorylation of normal liver mitochondria

Addition	Number of experiments	μ atoms Pi	μ atoms O_2	P:O
None	7	11.84 ± 1.17	4.32 ± 0.56	2.74 ± 0.17
Normal rat serum	7	9.53 ± 1.27	3.50 ± 0.39	2.73 ± 0.31
Sarcoma bearing rat serum	7	5.30 ± 1.35	3.26 ± 0.43	1.63 ± 0.43[a]

(± standard deviation; the values whose difference from the normal is statistically significant ($P < 0.01$) are indicated with [a])

Tab. II. ATPase activity in normal rat serum and in sarcoma-bearing rat serum. (The values are given as μg of P liberated in 15 min/mg of N, at 38°C, pH 7.4.)

	Number of experiments	Activity	mg N/ml
Normal rat serum	3	1.801	12.280
Sarcoma bearing rat serum	3	1.027	10.094

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Riassunto. Gli autori hanno esaminato l'influenza esercitata *in vitro* da parte del siero di ratti portatori di sarcoma Galliera sulla fosforilazione ossidativa in mitocondri di fegato normale. Dagli esperimenti effettuati risulta che il siero di portatore di tumore determina disaccoppiamento delle fosforilazioni dalle ossidazioni nei mitocondri di fegato normale. Gli autori, in riferimento a precedenti ricerche, discutono le probabili cause di questo fenomeno dovuto ad una sostanza o sostanze liberatesi dal tessuto neoplastico.

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Some Components of Adaptive Values of Heterozygous *Drosophila willistoni* from Irradiated Natural Populations

The adaptive value (w) is the integrative result of very numerous and complex biological properties contributing toward the genotypes' relative ability to perpetuate themselves throughout the generations.

In this work we attempt to measure some of the most important components of the adaptive values of irradiated *D. willistoni*. Wild individuals have been studied from a sample taken directly from an isolated wood, Capão A, which, in the course of a year, received about 420000 ($70000 \times 6F_1$) descendents of six irradiated samples (3 times 10000 r + 3 times 5000 r) by Cobalt 60 source of γ-radiation. As non-irradiated control, we used another isolated natural population of the same region (Eldorado, Rio Grande do Sul, Brasil)[1,2].

We studied the percentage of hatched eggs according to daily ovoposition, the *viability* as the total number of offspring from single couples in randomised culture vials[3] as true reproductive potentials of their genotypes; and the sterility as the mean frequencies of sterile matings[3]. These parameters of fitness were studied within a set of specified environmental conditions, of temperature, food supply[4] and technical details.

The most significant results can be briefly described as follows:

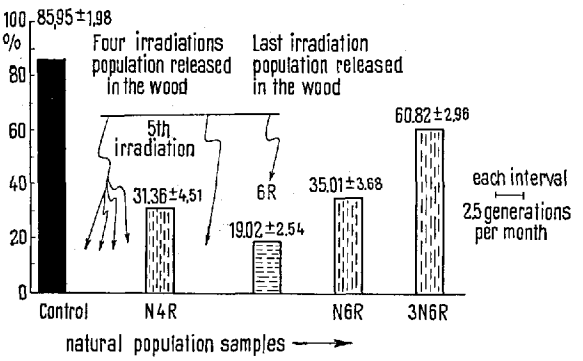
Percentage of hatched eggs was determined by counting the daily oviposition of inseminated females five days old, from the sixth to the tenth day, in all samples. The eggs were maintained at 25 ± 1°C at 95 to 100% of humidity. The counts of hatched (empty) eggs and the confirmation count of larvae were made 48 h after oviposition.

The first sample examined, $N4R_1$, was taken from Capão A one month (25 generations) after finishing a period of eight months during which four releases of 70000 F_1 of irradiated flies (respectively 10 + 10 + 10 + 5 Kr = 35 Kr) were made. In comparison with the control value (85.95 ± 1.98), the percentage of hatched eggs decreased significantly (see Figure). Two more releases of 70000 F_1 irradiated flies (5 + 5 Kr) were made during the next four months. The last irradiated population released, $6R_1$, analysed also exhibited a very low percentage of hatched eggs (Figure). The increase shown after about 3 months (7.5 generations) by the N6R is most significant, however, in the 3N6R sample (3 months later), a significant three-fold increase occurred (Figure). Even this increase did not restore the level of the natural non-irradiated control population.

A total of 3280 eggs were examined in these tests.

Viability was measured by the mean number of offspring produced by fertile simple pairs of flies[3]. Control and irradiated natural populations, and the last laboratory irradiated sample released, were run in five simultaneous series of experiments:

($6R_1$ < Control A), ($N6R_1$ < Control E_1), ($2N6R_1$ < Control E_2), ($3N6R_1$ = Control E_2) ($6N6R_1$ = Control E_4)



% of hatched eggs of *Drosophila willistoni* control and irradiated natural populations.

as can be seen in the Table. The very significant decrease of viability of the $6R_1$ (−17%) was followed by the irradiated natural population samples ($N6R_1$ −21%; $2N6R_1$ −16%) until the recuperation attained by the $3N6R_1$ 6 months (15 generations) after the last release; and this was further confirmed by the $6N6R_1$ 14 months (approximately 35 generations) after the 6R release.

A total of 36854 among the experimental, and 24183 individuals in the control cultures, has been counted.

The sterility defined as the number of simple pairs of flies that do not produce offspring[3] gave the interesting information that in natural population they are eliminated as equivalent of dominant lethals. Only the F_1 of the 6R showed a significantly higher percentage than 37.6%. All samples from nature are around a mean value of 3.5% sterile pairs.

Some irradiated experimental population of *D. melanogaster* increased their fitness in comparison with non-irradiated[3] which seems to be dependent on population size and the advantage of induced mutants in heterozygous condition. The irradiated natural populations of *D. ananassae* near the atomic bombed Marshall Islands[5,6] showed an extreme depression in some biological properties. The flies heavily irradiated by direct and fallout radiations from atomic tests, especially in March 1954 (Bikini islands), showed a greater load of deleterious mutants and low fitness values, expressed in egg development, in comparison with much less irradiated natural

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