mechanism to the inhibition remains for future investigation.

In the presence of ions of large atomic number such as I^-, the collisional perturbation effect of spin-orbital coupling in the π-electron orbitals of RF by I^- favours intersystem crossing[5] in the excited RF (high Z atom effect[2,9]). The resulting shortening of the lifetime of RF^b explains the inhibition. Furthermore, I^- has a donor activity, and a charge transfer from I^- to RF produces the RF^- radical[10] which is non-fluorescent and non-phosphorescent. This kind of charge transfer may reduce the production of RF^b by diminishing the overall quantum yield of photoinactivation.

PPD is a strong quencher of RF^b and is photo-oxidized in the presence of O_2. The possible mechanism of deactivation of RF^b may be a charge transfer from PPD to RF^b.

Another type of inhibition mechanism can be seen in the inhibition reaction by T_r. T_r has a considerable ability to form a charge-transfer complex[11] ($RF^- \cdot T_r^+$) (non-fluorescent and non-phosphorescent) with RF in the ground state. The overall quantum yield of production of RF^b is reduced by the formation of this complex to some extent. T_r is easily oxidized by the photosensitization of RF. Since we have no evidence that RF^b is selectively quenched by T_r, it may be natural to consider that a preferential destruction of the reactive oxygen is the main mechanism of inhibition.

The inhibition mechanisms mentioned above are considered to be typical. At low inhibitor concentration, only one of the factors[12] is sensitive and R_0/R-C relation is linear. However, at high inhibitor concentration, several factors may be mixed up; deviations from the linear relation between R_0/R and C may then occur. Further details will be described in a future paper.

Zusammenfassung. Durch Gegenwart von Co^{++} oder J^- oder Tryptophan oder *p*-Phenylendiamin wird die durch Riboflavin sensibilisierte Photoinaktivierung der Taka-Amylase A stark gehemmt. Die möglichen Mechanismen der Hemmungswirkung dieser Inhibitoren werden diskutiert.

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Institute of Biophysics, Faculty of Agriculture, Kyushu University, Fukuoka (Japan), August 3, 1966.

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[12] The factors mean the quenching of RF^a and RF^b and the destruction of the reactive oxygen in the oxidation process, by the inhibitors.

Effects of Histones on Embryonic Cells

Since histones are intimately associated with the genetic material, DNA, they are suspect as regulators of gene activity[1-4]. Assuming that foreign histones might reach the nucleus of a cell exposed to a histone solution one might be able to demonstrate specific effects on gene loci or the gene activating mechanism. Such effects have been proposed for treated embryonic organs[5,6] and for whole embryos when treated with preparations containing histones at various concentrations[7-9].

We were prompted, therefore, to examine the effects of histones on organ cultures of chick embryonic skin, and its ectodermal organ derivative, the down feather. 2 histone preparations were used: whole calf thymus histone (CTH) and a diazotized sample of the same (DCTH). Unfractionated calf thymus histone was prepared from the ethanol-washed deoxyribonucleohistone by extraction with 0.2 *N* HCl[10]. A sample of this histone was diazotized and coupled with sulfanilic acid[11] in order to diminish its positive charge. If CTH is added to an extract medium a precipitate is immediately formed. Therefore, 7-day-old chick embryo back skin was dissected into bilateral halves, each 2 mm^2, rolled inward so that the mesenchyme portion was on the outside, and incubated for periods up to 1 h in Tyrode solution in a shaking water bath. To $^1/_2$ of each bilateral pair CTH was added to the incubation medium in concentrations ranging from 10 $\mu g/cm^3$ to 5 mg/cm^3. After the incubation, the skin pairs were cultured on stainless steel rafts in Falcon culture dishes on an embryo extract (20%)-Tyrode solution medium for periods of up to 6 days. Most of the tissues exposed to the highest CTH concentration of 5 mg/cm^3 became necrotic (Figure 1). Those tissues exposed to a

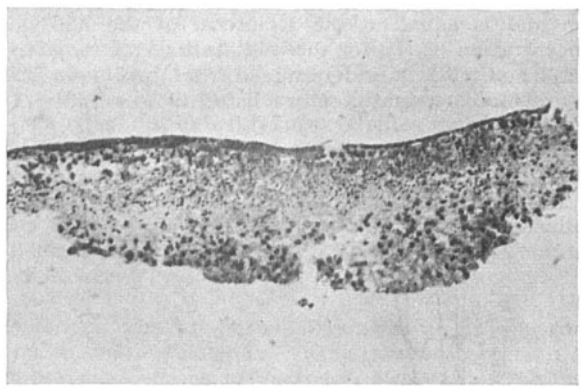

Fig. 1. Chick embryo skin grown in organ culture for 3 days after incubation in 5 mg/ml CTH for 1 h.

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lesser concentration proceeded to grow and differentiate, and did not differ from control tissues (Figure 2). It appeared that the number of necrotic cells in a tissue was proportional to the concentration of histone to which it was exposed. Histological sections of the skin prepared from zero time to 6 days in culture demonstrated no increase in histone in viable cells by Fast Green staining[12].

DCTH was added directly to the culture medium since its addition did not effect a precipitate. Concentrations varied from 10 $\mu g/cm^3$ to 500 $\mu g/cm^3$ with no demonstrable effects.

We next wished to determine the LD_{50} of histone-treated embryonic cells. In 3 separate experiments, the results of which were identical, 8- and 14-day-old whole chick embryos, and 14-day-old chick embryo brain alone, were minced, trypsinized and gassed with 5% CO_2 in air, and incubated for 1 h to produce a cell suspension. After incubation the suspension was flushed gently to further separate the cells, packed by gentle centrifugation and washed 3 times with 20 volumes of Tyrode solution or Tyrode solution with trypsin inhibitor added. Both methods demonstrated the same results. After a final suspension, 2 aliquots, one serving as control and the other to be treated with CTH, were gassed and incubated for periods of up to 1.5 h. CTH was added to 1 aliquot in concentrations of 10–50 $\mu g/cm^3$.

The Table shows the LD_{50} established at 25 $\mu g/cm^3$. At 50 $\mu g/cm^3$ all cells were dead (Figures 3 and 4). The lethal effect occurred at zero time and did not increase with time of incubation. In contrast, incubation of the cells with 500 $\mu g/cm^3$ of DCTH for 1 h had no effect.

To study the effect of histones on differentiation, 8-day-old chick embryo livers and back skins were each dissociated and prepared as cell suspensions, as before. One group of each was incubated with CTH in concentrations ranging from 1–25 $\mu g/cm^3$ and control and treated groups incubated for periods of up to 1 h. Aliquots from each suspension were then reaggregated as hanging drops on glass cover slips in a CO_2 incubator for up to 3 days. After this time, some reaggregated organs were transferred to organ culture and incubations continued for 3 additional days. Those tissues having received 25 $\mu g/cm^3$ of CTH showed a corresponding level of necrotic cells, and the organ failed to reconstruct properly. Below this concentration the tissues reconstructed in the same way as controls, and no morphological or histological differences were observed. Fast Green staining was not increased in viable cells.

The above results are consistent with the reported toxicity of CTH and rat leukemia histone by HNILICA and HOLOUBEK[13] for new-born rats. The fact that the lethal effect on the embryonic cells tested occurred instantaneously and did not increase with time would seem to indicate a destructive effect on the cell membranes. Such effects have been recently suggested by BLAZSEK and GYERGYAY[14].

Developmental anomalies reported in the literature, therefore, may be due to selective death of embryonic cells. CTH also penetrates poorly into embryos or tissues in which mucopolysaccharides were abundant, presumably because of interaction of the highly charged compounds of the tissue matrix with the strongly basic histones. DCTH, with no positive charge, however, had no destructive effect on the cells, although no unusual

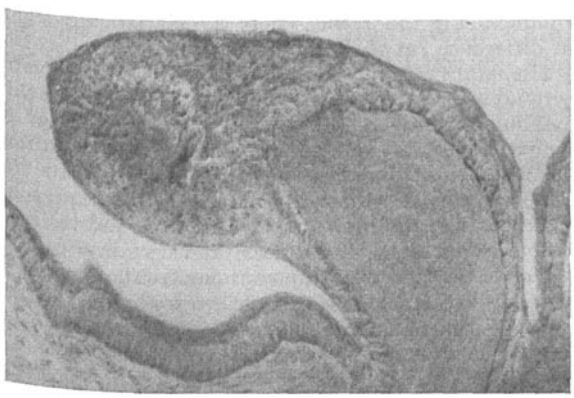

Fig. 2. Control chick embryo skin and down feather organs grown in organ culture for 3 days. Compare with Figure 3.

Eosin dye exclusion tests based on hemocytometer counts on incubated cells in the presence of CTH at timed intervals

% treated cells viable / % control cells viable				
Concentration of CTH	15 min	30 min	60 min	90 min
10 $\mu g/ml$	0.99	1.0	0.97	0.98
25 $\mu g/ml$	0.52	0.50	0.51	0.51
50 $\mu g/ml$	0	0	0	0

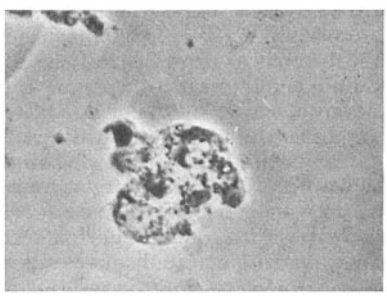

Fig. 3. Embryonic cells from 8-day-old chick exposed to 50 $\mu g/ml$ of CTH.

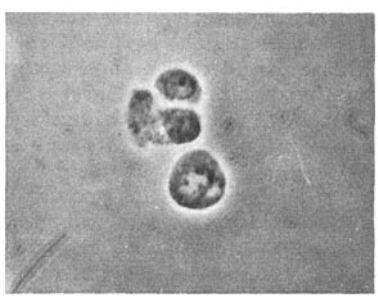

Fig. 4. Control embryonic cells of 8-day-old chick. Compare with Figure 1.

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effects from the treatment with this compound were observed either in cell suspension or organ culture[15,16].

Zusammenfassung. Der Einfluss vom Kalbsthymus Histon (CTH) und seines diazotierten Derivatives (DCTH) auf Vitalität und Zelldifferenzierung verschiedener Gewebe des Hühnerembryos wurde geprüft. Die LD_{50} für CTH wurde bei 25 µg/ml der Zellsuspensionen festgestellt. Es wurden keine bedeutenden Änderungen in der Differenzierung der embryonalen Gewebe in Kulturen bei sublethalen Konzentrationen gefunden.

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[16] The authors wish to thank Miss PAMELA PHILLIPS for her technical assistance.

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Variation in Intestinal Serotonin Levels, and the Effect of Reserpine in Sprague-Dawley Rats from Different Sources

Approximately 60% of the total body serotonin in rats is present in the gastrointestinal mucose[1]. Consequently, this tissue has been used frequently to study the effects of various drugs on serotonin metabolism. In earlier communications it has been reported that fasting[2] and prior exposure of the rat to anesthetic agents[3] adversely affect bowel serotonin. Of greater importance is that both sex and strain differences of statistical significance exist in bowel serotonin levels in rats of Sprague-Dawley origin from different suppliers[4]. Because of this latter finding it would seem important to identify the strain of the experimental animal, and also the laboratory of origin. It seems reasonable to assume that the failure to reproduce published experiments may depend in part (even when extreme care is observed to follow reported techniques) on strain differences in drug metabolism or response. In vivo experiments are subject to the intrusion of many variables, such as diet, alterations in room temperature or housing, seasonal or circadian influences etc. While investigating the effect of reserpine on bowel serotonin metabolism one shipment of rats was found to respond poorly to the depleting effect of this amine. Because no satisfactory explanation could be found to account for this finding, the source of the animals was investigated. The standing order of rats shipped every 2 weeks, routinely came from the Charles River laboratories' breeding shed I. However, on this occasion rats had been shipped from breeding shed IV. Both of these sheds contain colonies of randomly-bred rats of Sprague-Dawley origin[5], but maintained separately and autonomously. It is generally realized that animals of a second species may respond differently to drugs or experimental procedures. However, of equal importance is the fact that animals of the same species, and from the same supplier, may exhibit variations in drug response. The purpose of this communication is to emphasize the fact that animals of the same species may differ greatly, and that this difference may introduce an important source of biological error.

Male Sprague-Dawley rats weighing between 200–300 g from the Charles River breeding sheds I and IV were used. Animals were kept in colony cages and fed commercial Purina rat chow with a tryptophan content of 0.22%. To avoid any possible circadian influences on serotonin levels (to date only reported for rat brain[6]), rats were always killed between 0800 and 1000 on the day of assay. Rats were decapitated by a small animal decapitator (Harvard Apparatus Co. Inc., Dover, Mass.), without prior exposure to anesthesia. Five to eight $^1/_2$ inch segments of jejunum commencing 6 inches distal to the pyloric sphincter were taken from each rat. Details of our method of mucosa muscle separation and spectrophotofluorometric analysis have been reported previously[4]. Reserpine (5 mg/kg) was injected i.p. in one dose. Rats killed at 0 time received i.p. saline.

Results were compared statistically using the t test and are indicated in the Table. There is a statistically significant difference between the values at 0 time in rats from the 2 separate strains ($p < 0.025$). Although a depletion of jejunal serotonin develops following reserpine, reaching a maximum at 4 h in both groups, the fall is greater and more prolonged in those rats from shed I. The maximum fall in serotonin concentration is 54% for the rats from shed I, and 19% for the rats from shed IV. The reason(s) for this discrepancy between the untreated and the reserpine pretreated rats is not clear, and certainly calls for further study. The literature contains conflicting reports of the effect of reserpine on bowel serotonin levels. In the rat, ERSPAMER has shown that minimal serotonin depletion occurs following reserpine[7], while MORAN and WESTERHOLM[8] report no effect on serotonin levels following reserpine. SANYAL and WEST[9] on the other hand report depletion of rat intestinal serotonin by pretreatment with reserpine; however, these authors give no actual values. This divergence of opinion may in part be

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