tion of collagen was controlled by growth promoters and in this way collagen production would be directly related to growth requirements.

The apparent importance of AA in other areas associated with growth, e.g. in the formation of mucopolysaccharide[33], the maintenance of ribosome orientation[34] and in the removal of histone from inducible chromatin[35], would also ensure that unrestrained growth did not accompany 'ascorbate' deficiency. Thus scurvy is mainly characterized by the lack or faulty development of structural substances and the inability of cells to differentiate although an increase in nuclei, respiration and enzyme activity[36] have also been observed during the development of scurvy.

The reason for the high concentration of AA in the adrenal glands has not been established but it has been suggested that it may play a primary part in the control of steroidogenesis through the adrenal hydroxylase system[37]. Stress in animals leads to the hydroxylation of adrenal steroids and secretion of these and DHA from the adrenal glands[37]. The increase in the level of circulating DHA is in agreement with the reduction in the mitotic rate associated with stress and would also explain the diurnal mitotic rhythm found in a number of animal tissues[38]. Thus during sleep stimulation of the adrenal glands is less, the level of DHA falls and the mitotic rate increases.

The above examples of existing evidence support the hypothesis that DHA is an important inhibitor of cell growth and division and suggest that many aspects of the extensive literature on 'ascorbate' may be rationalized on this basis.

Résumé. L'acide dehydroascorbique peut agir comme un inhibiteur de la croissance et de la division des cellules, suivant une théorie générale proposée par SZENT-GYÖRGYI[1]. Des exemples tirés d'autres témoignages soutiennent ce point de vue.

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STUDIORUM PROGRESSUS

Radiation Induced Avoidance Behavior Transfer by Brain Extracts of Mice

Since 1951 we have been studying the effects of X-radiation on brain tissue of mammals[1]. In recent years our interest has been concerned with cellular effects, as well as radiation induced behavioral changes. That the effects of radiation are not confined to their direct effects on cells nor to the immediate period of exposure to the radiation has long been known by students of experimental radiology. Hiroshima and Nagasaki are prime examples, also this axiom was re-emphasized through the study of HOLSTEN et al.[2] on plant cells and especially their growth media, while their results for *Drosophila melanogaster* were less definitive.

A considerable literature has accumulated in recent years concerning the effect of ionizing radiation on behaviour, especially on the radiation-induced behaviour involved in conditioned avoidance of a distinctive taste substance (saccharine sodium)[3,4]. The sweet solutions are offered simultaneously to the mice with plain tap water before, during, or after the animals are exposed to radiation. A concentration of 0.1% by weight of saccharine has been found adequate to establish taste preference of this solution to that of tap water, and the conditioned stimulus is thus established.

In this report we are extending our past studies[5–8] concerning the direct and indirect effects of X-radiation on conditioned avoidance behaviour. The work of many laboratories, including our own, has established that mice prefer saccharine sodium solution to plain water. However, when the saccharine solution is offered just before the animals are exposed to ionizing radiations, post-irradiation avoidance is induced. The physiological mechanism of saccharine avoidance behaviour has not yet become clear. Still unanswered are the questions: is the effect caused by pathological changes in the irradiated animals, or are changes induced in the taste perception mechanism? Is it strictly a prolonged disturbance of some of such mechanisms or is an impairment of memory function involved? Furthermore, what is the duration of these effects, and how are the basic cell biological mechanisms altered?

Most memory transfer studies date back to 1960 when MCCONNELL[9,10] found that untrained flatworms would learn more quickly when injected with ribonucleic acid from trained flatworms. Several other scientists have postulated that transfer of knowledge might be accomplished by feeding an animal which has learned some particular task to untrained animals or by grinding up the brain of one animal and injecting it into another.

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However, other experiments using Planaria and also mice did not confirm this transfer of knowledge[11–14]. In fact BYRNE[15] among 23 scientists from 7 different countries signed a letter to Science that stated their inability to confirm this learning transfer by injection of brain extract from trained donors.

JOHN[16] has reported that animals who receive a small amount of potassium injected directly into the brain before each learning experience learn as rapidly as the most rapid learners in a normal population. Conversely, animals receiving minute injections of calcium directly into the brain before each learning experience displayed extremely slow acquisition of the responses. Thus the rapidly learning or the slowly learning limits of a normal population can be displaced by minute injections of chemicals into the brain fluids. Many parts of the brain participate in the memory for previously acquired responses.

Many questions raised by above-mentioned reports and the ones concerned with the controversial findings in Planaria[17,18] and the clear-cut results of HOLSTEN, SUGII, and STEWARD[2] with plant tissues, but not with *Drosophila*, remain unanswered at present.

Though being aware of the controversial nature of the undertaking we decided to test by transfer of brain substance for transmission of the X-irradiation effect in mice trained to drink Na-saccharine solution before irradiation. It appeared that this experimental procedure was particularly suitable and reliable for such a task. Furthermore, it was hoped that should the results be positive new light would be shed on the mechanism causing saccharine avoidance behaviour. It is the purpose of this report to present our findings on the effects of brain substance injection from irradiation-saccharine avoidance conditioned mice into normal control animals.

Methods. Male DF_1 mice of approximately 50 days of age were used throughout all experiments. One week to 10 days were permitted for the animals to recover from shipment and adapt to their new environment before the start of each run.

The mice were randomly distributed into groups as follows: (A) 2 main groups, namely 'donors' and 'recipients' of brain substance. (B) These 2 groups were then divided up into 6 sub-groups:

Donor mice	Recipient mice
(1) Irradiated and saccharine-preference conditioned.	Not saccharine preference-conditioned.
(2) Irradiated and not saccharine-preference conditioned.	Not saccharine-preference conditioned.
(3) Irradiated and saccharine-preference conditioned.	Saccharine-preference conditioned.
(4) Irradiated and not saccharine-preference conditioned.	Not saccharine-preference conditioned.
(5) Not irradiated, no animal brain, Ringer solution only.	Not saccharine-preference conditioned.
(6) Not irradiated, not saccharine-preference conditioned.	Not saccharine-preference conditioned.

Further details as to number of mice in each group, radiation dosage, etc., are presented in Table I.

The conditioning of the mice was usually established after about 1 week to 10 days. This was accomplished by offering 1 bottle of regular tap water and 1 bottle containing 1% (per weight) sodium-saccharine solution. All animals were handled and observed under identical conditions, and the liquid consumption determined daily at the same time. The control animals (unconditioned) were provided with tap water only. The 'donor' animals were deprived of all liquids 24 h before irradiation but then shortly before radiation treatment saccharine solution alone was offered for 30 min.

Total body irradiation was performed with a Picker X-ray machine at 220 KV, 20 mA. The HVL was 0.5 mm Cu after additional filtration of 0.25 mm Cu. Target to mouse distance was 50 cm and the field size 20×20 cm. Up to 6 control and experimental 'donor' mice could be irradiated with 400 rads under these conditions. For uniform dose distribution the plastic animal container was rotated at 3 rpm.

For the transfer of brain substance the following methods were employed: the brains of irradiated (except experimental run 5 and 6) either saccharine-preferring or control (not having been exposed to saccharine solution) mice were removed in their entirety from the skulls of the animals. Usually 3 brains per group were used with the exception of the first few runs where only 1 brain each was removed from either group. The 'donor' animals were killed by spinal cord severance in the cervical region about 10–30 min after X-ray exposure. Each group of brains (saccharine-preference conditioned and untrained controls) were ground up separately in 10 ml sterile physiological Ringer's solution in a sterile mortar at a temperature of 3–5 °C. The brains were ground into a fairly homogeneous tissue suspension by 10 min of grinding and 0.03 ml (0.01 ml in the first run) was injected into the right posterior quadrant of the brain of unirradiated mice. For the intracerebral injections 1 ml tuberculin syringes and 28 gauge $^1/_2$″ needles were employed. The needles were provided with a little 'lucite' disk through which only about 3 mm protruded at the tip. This insured a more uniform depth of insertion into the brain.

Results. The observation of daily water and saccharine consumption of the brain substance recipient animals lead to the following findings:

(1) Naive mice receiving 0.3 ml of a brain suspension from irradiated and saccharine-preference trained donors (see Experiment 1 in Table I) showed a consistent refusal to consume saccharine solution in any significant amounts. The regular unsweetened tap water was definitely preferred and the difference between the amounts of water consumption and that of saccharine was statistically significant. The aversive tendencies are not permanent and preference for the saccharine solution apparently increases after some days but at a very much slower rate than observed normally.

(2) In contrast to the above group it could be shown as a control experiment that injection of brain suspensions

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from untrained (naive) but irradiated mice into groups of naive control animals (see Experiment 2 in Table I) produced no avoidance behaviour. The animals acquired saccharine preference as rapidly as any normal group of mice, which had never been exposed to any irradiation or any other type of treatments. These 2 main results are presented in Figure 1 where the sets of curves in 1 and 2 correspond with (1) and (2) of above description of experimental findings and Tables I and II. The error limits indicated at each point of the curves are the standard errors. Intercomparison of the 4 curves as to whether or not the observed differences are real, revealed that such differences are highly significant on a $p > 0.001$ confidence level. When however the saccharine consumption of naive control animals injected with irradiated and conditioned brain substance is compared with the water consumption of untrained mice having received an injection from irradiated but non-conditioned mice. No great difference is found and the t-test indicated that any existing differences are barely significant at the p 0.05 level.

(3) In Figure 2 curve sets a and b (Table I and II, Experiment 3 and 4) represent data from identical experiments as those described above except that in these instances both recipient groups consisted of saccharine-preference trained animals. Animals in these groups receiving brain suspension from irradiated and conditioned mice (Figure 2a) showed only a slight saccharine avoidance and then recovered this preference within about 9 days. Changes in water consumption were inconclusive.

Repetition of the experiment (run simultaneously) with irradiated but naive brain injections into conditioned mice gave the results shown in Figure 2b. There was only a momentary drop in saccharine consumption and practically no change in that of water.

It seems that saccharine-preference conditioned (trained) recipient mice respond to a much smaller degree to above test procedures than animals which have not yet learned to prefer the sweet solution.

(4) Experiments 5 and 6 (Table I) were control experiments which were performed to establish if the traumatic experience of the injection alone (Ringer solution only, Figure 3a) or material from an unirradiated brain (Figure 3b) could induce avoidance behaviour. In neither experiment could this effect be produced.

Discussion. The mechanism of radiation induced avoidance response is at present not sufficiently known.

Table I. Findings

Experiment	X dose rad	Donor mice	No. of donors	Recipient mice	No. of recipients	Saccharine consumption	H_2O consumption
1	400	saccharine-conditioned	1–3	*not* saccharine-conditioned	49	*avoided*	increased
2	400	*not* saccharine-conditioned	1–3	*not* saccharine-conditioned	46	*not* avoided	decreasing
3	400	saccharine-conditioned	1–3	saccharine-conditioned	20	slight avoidance and fast recovery	no significant change
4	400	*not* saccharine-conditioned	1	saccharine-conditioned	9	momentary drop but no significant change	no change
5	0	Ringer solution only	0	*not* saccharine-conditioned	11	*not* avoided	decreased
6	0	*not* saccharine-conditioned	1	*not* saccharine-conditioned	11	*not* avoided	decreased

Table II. Comparison of liquid consumption

Experiment	Donor	Recipient	Consumption of	Compared with	Experiment	Donor	Recipient	Consumption of	Consumption differences and remarks[a]
1	saccharine-conditioned	*not* conditioned	saccharine		1	saccharine-conditioned	*not* conditioned	H_2O	$p > 0.001$ $H_2O_{(1)} >$ saccharine$_{(1)}$
2	*not* conditioned	*not* conditioned	saccharine		2	*not* conditioned	*not* conditioned	H_2O	$p > 0.001$ saccharine$_{(2)} > H_2O_{(2)}$
2	*not* conditioned	*not* conditioned	saccharine		1	saccharine-conditioned	*not* conditioned	saccharine	$p > 0.001$ saccharine$_{(2)}$ > saccharine$_{(1)}$
2	*not* conditioned	*not* conditioned	H_2O		1	saccharine-conditioned	*not* conditioned	H_2O	$p 0. > 001$ $H_2O_{(1)} > H_2O_{(2)}$
1	saccharine-conditioned	*not* conditioned	saccharine		2	*not* conditioned	*not* conditioned	H_2O	not significant $p \leqslant 0.05$ saccharine$_{(1)} \simeq H_2O_{(2)}$
1	saccharine-conditioned	*not* conditioned	H_2O		2	*not* conditioned	*not* conditioned	saccharine	$p > 0.001$ saccharine$_{(2)} > H_2O_{(1)}$

[a] Indices in parentheses, e.g. (1) and (2) which refer to experiment 1 or 2.

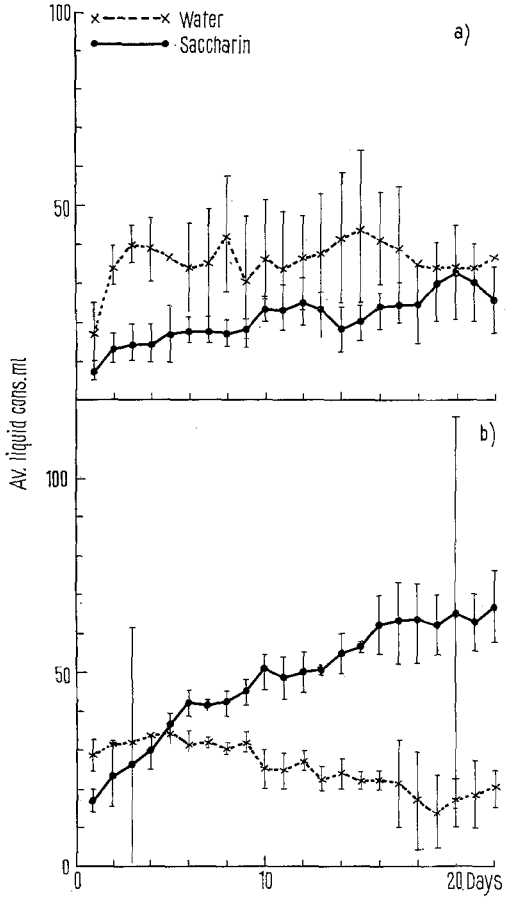

Fig. 1. (a) Saccharine and H_2O consumption of 'naive' (not irradiated) mice injected with brain suspension from saccharine preferring and irradiated donors. Saccharine solution is avoided. (b) Saccharine and H_2O consumption of 'naive' (not irradiated) mice injected with brain suspension from 'naive' but irradiated donors. Saccharine solution is preferred.

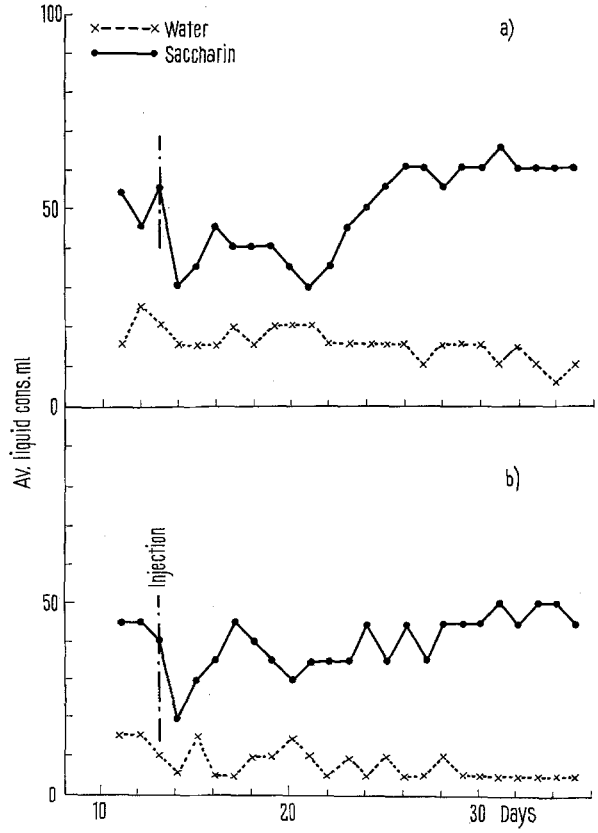

Fig. 2. (a) Saccharine and H_2O consumption of 'trained' (saccharine preferring) mice (not irradiated) injected with brain suspension from saccharine preferring and irradiated donors. Saccharine solution consumption only momentarily reduced. (b) Saccharine and H_2O consumption of 'trained' (saccharine preferring) mice (not irradiated) injected with brain suspension from 'naive' but irradiated donors. Saccharine solution consumption only momentarily reduced.

Moreover, the fact that such behaviour can be transferred from one higher animal to another seems to indicate that either directly through radiation or indirectly by associating an unpleasant stimulus such as X-irradiation with a learned process (conditioning) a more than momentary product is created in the animal body. The possibility of transferring such a product as we may have achieved in our experiments suggests several possibilities. For instance, very specific substances can be produced in increasing amounts as a better response is learned by an organism, as for example in our experiments where the conditioning for saccharine preference was used. This seems to be indicated by the increasing consumption of saccharine over a certain time period. It is suggested by our results that a substance produced by the animal contains some information in its molecular structure and in a certain codified form. This molecular structure if subjected to X-irradiation is possibly altered and consequently also its coding or, what is more likely, a second specific product is formed by interaction of ionizing radiation with certain molecules which then in some manner form a new information structure (learning to associate saccharine drinking with an unpleasant stimulus). This new 'code structure' can then be introduced into inexperienced (naive) animals which in this manner now have acquired instructions to avoid saccharine.

One of the further steps of our experimentations is to investigate whether such 'products' can be produced only in the brain or also in other body parts. Then the next sequence of research will be to explore factors influencing short and long term memory.

Since mice injected with irradiated brain tissue from conditioned animals show an immediate aversion to saccharine (Figure 2a) it is suggested that changes in protein synthesis, and consequently in RNA molecules, are not the only controlling factors in above-described processes. This line of thought is supported by the results of BARONDES et al.[19–21] which indicate that protein synthesis is not necessary for short-term memory but is required for long-term memory. Destruction of long-term memory or suppression of protein synthesis by drugs such as 'puromycin' should show up in the form of a short saccharine aversion period followed by increased consumption of the sweet solution similar to observations

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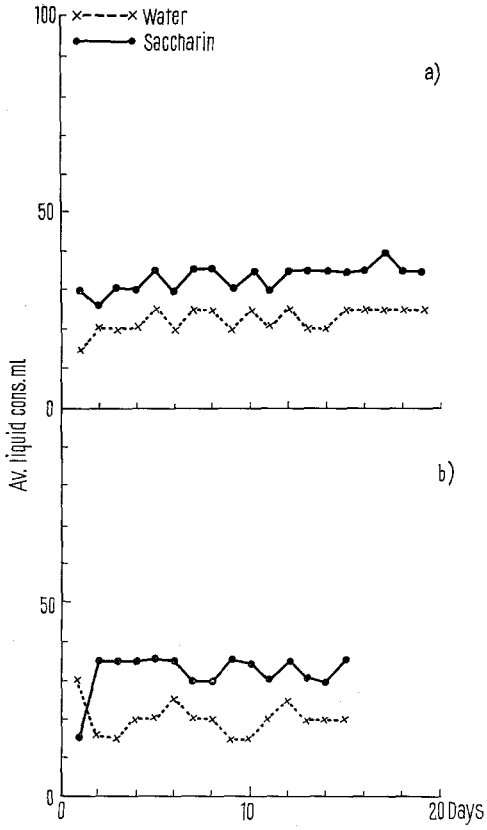

Fig. 3. (a) Control experiment: Saccharine and H_2O consumption of 'naive' (not irradiated) mice after injection of Ringer solution only. (b) Control experiment: Saccharine and H_2O consumption of 'naive' (not irradiated) mice after injection with brain substance from a 'naive' and unirradiated donor.

made with avoidance behaviour of mice to which irradiated saccharine solution was offered.

Should such future investigation be successful, one could perhaps attempt to identify the radiation-affected molecular structures[22]. Although these thoughts are conjectural, they seem to be part of a logical system in clarifying some very puzzling aspects of memory synthesis and radiation-mechanisms as well[23].

Zusammenfassung. In «naiven» Mäusen werden Verhaltensveränderungen nach Injektion kleiner Mengen von Gehirnmaterial aus Saccharin bevorzugenden und röntgenbestrahlten Mäusen beobachtet. Es trat Vermeidung der Saccharinlösung ein, und zwar ähnlich wie sonst in «trainierten», Saccharin bevorzugenden Tieren nach der Bestrahlung.

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Kinetik der hepatischen Farbstoffaufnahme von Indocyaningrün. Einfluss von Bilirubin und Natriumglykocholat

Untersuchungen über die Kinetik der hepatischen Ausscheidung von Farbstoffen haben die Kenntnisse der Leberphysiologie sehr bereichert. Als Farbstoffe dienten vor allem Bromsulphalein, Bengalrot und Indocyaningrün (ICG). Von diesen weist ICG die geringste extrahepatische Ausscheidung auf[1].

Nachdem ICG von Fox et al.[2] zur Bestimmung des Herzzeitvolumens in die Kreislaufdiagnostik eingeführt worden war, fand es bald Anwendung zur Bestimmung der Leberdurchblutung nach dem Fickschen Prinzip[3] und zur Prüfung der Leberfunktion[4–10]. Als besonderer Vorteil von ICG erwies sich dabei die Möglichkeit, seine Clearance mit Hilfe eines Ohrdensitometers unblutig zu registrieren[8, 9].

Zur Leberfunktionsprüfung mittels ICG-Clearance wurden bisher sehr unterschiedliche Dosierungen des ICG angegeben[4–10]. Tierexperimentelle und klinische Studien haben jedoch gezeigt, dass die Empfindlichkeit der ICG-Clearance als Leberfunktionstest von der Farbstoffdosis abhängt. Während nämlich die Clearance kleiner ICG-Dosen (0,5 mg/kg) vor allem von der Leberdurchblutung abhängt und ein relativ unempfindlicher Parameter der Leberfunktion ist, stellt die Clearance grosser ICG-Dosen (5 mg/kg) einen sehr empfindlichen

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