Effects of Ionizing Radiation on the Nervous System

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EFFECTS OF IONIZING RADIATION ON THE NERVOUS SYSTEM

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ON THE NERVOUS SYSTEM
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FOREWORD

One of the most important developments in radiobiology in recent years has been a change in the concept of the ability of the nervous system to withstand the effects of ionizing radiation. Until recently it had been generally believed that the tissue of the nervous system was especially resistant to radiation and that any kind of damage to it could occur only at very high radiation doses. Over the past few years, however, there has been increasing evidence to show that the nervous system responds to even small doses of radiation and that this response may often be associated with some form of radiation damage.

To discuss the most recent findings, the International Atomic Energy Agency sponsored a Symposium on the Effects of Ionizing Radiation on the Nervous System, which was held in Vienna from 5—9 June, 1961. About 70 leading experts in the field attended, representing 20 countries, and 34 papers were presented.

In the work described, all aspects of the response to radiation exposure of both peripheral and central nervous systems were discussed. Particular emphasis was given to the reaction of the central nervous system, as knowledge of its reaction to irradiation may provide additional criteria for the establishment of safety codes for nuclear operations. A great deal of research work on radiation effects on the nervous system has been done in some countries and this Symposium provided an occasion for a detailed review of the results.

There is no doubt that the effects of ionizing radiation on the nervous system rank among the foremost subjects of interest to all those working in the nuclear energy field, and it is hoped that the information now made available in these Proceedings will be of value to them.

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PERIPHERAL NERVOUS SYSTEM

RADIOBIOLOGY OF NERVOUS RECEPTORS

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Abstract—Résumé—Аннотация—Resumen

Radiobiology of nervous receptors. The functional role of membranes, cellular and extracellular, are described, and those membrane properties which may contribute to the explanation of the action of ionizing radiation on excitable tissues, are discussed in more detail. Particular attention is given to the influence of radiation on polarization, ionic permeability and the liberation of neurohormones. Early phenomena of the radiation syndrome, which are likely to be caused by the freeing of neurohormones, are then discussed. Publications demonstrating the radiation sensitivity of photoreceptors are chronologically reviewed. The possible causal significance of the liberation of neurohormones is discussed.

Radiobiologie des récepteurs nerveux. L'auteur décrit le rôle fonctionnel des membranes cellulaires et extracellulaires et examine de manière plus approfondie celles des propriétés des membranes qui peuvent aider à expliquer l'action des rayonnements ionisants sur des tissus excitables. Il étudie en détail l'influence des rayonnements sur la polarisation, la perméabilité ionique et la libération des neurohormones. Il examine ensuite les premiers phénomènes du syndrome d'irradiation qui sont susceptibles d'être produits par la libération de neurohormones. L'auteur passe ensuite en revue par ordre chronologique les publications traitant de la radiosensibilité des photorécepteurs. Il étudie l'importance possible de la libération de neurohormones.

Радиобиология нервных рецепторов. Описывается функциональная роль клеточных и внеклеточных мембран и более подробно обсуждаются те свойства мембраны, которые могут способствовать объяснению действия ионизирующей радиации на возбудимые ткани. Особое внимание уделяется влиянию радиации на поляризацию, ионную проницаемость и выделение нейрогормонов. Затем обсуждаются ранние симптомы радиационного синдрома, которые, вероятно, вызываются освобождением нейрогормонов. В хронологическом порядке рассматриваются публикации, демонстрирующие чувствительность к излучению фоторецепторов. Обсуждается возможное значение выделения нейрогормонов.

Radiobiología de los neurorreceptores. El autor describe la función de las membranas celulares y extracelulares y examina detalladamente aquellas propiedades de las mismas que pueden contribuir a explicar la acción de las radiaciones ionizantes sobre los tejidos excitables. Estudia con particular detenimiento la influencia de las radiaciones sobre la polarización, la permeabilidad iónica y la liberación de las neurohormonas; seguidamente examina los fenómenos iniciales del síndrome de irradiación, que pueden tener su origen en la liberación de neurohormonas. Presenta una reseña cronológica de las publicaciones que tratan de la radiosensibilidad de los fotorreceptores. Por último, estudia el posible significado de la liberación de neurohormonas.

For a short survey it appears useful to start with a few words on membranes, cellular and extracellular. "Membrane" is taken in the functional sense: a microbarrier possessing some impermeability towards various types of molecules or ions.

Relative impermeability in aqueous media can be obtained by insolubility, or by structure; the first type demands the presence of a more or less continuous layer

of fatty substances, generally covered on the polar sides with a very thin protein layer (Danielli type); the second arises from a macromolecular gel with potency of "gel filtration". In many membranes both types are present. If cytoplasm contains two-phase systems, a fractionation of macromolecules in this way is another possibility, ALBERTSSON [1].

For sharp barriers to small molecules or ions (inorganic ions, neurohormones, coenzymes) only the fatty type can serve. And, though the phenomenon has not stimulated recent interest, I would like to refer here to the possibility of phase reversal in thicker membranes, e. g. by Ca^{++} ions [2].

Ussing's flux equation is valid for passive diffusion of ions through any membrane [3]:

$M_{in}/M_{out} = (c_o/e_i)$ exp. $(\chi FE/RT)$, where
M_{in} is the inward flux, M_{out} the outward flux,
c_o the ion concentration in the outside solution,
c_i in the inside solution,
E is the potential difference between outside and inside solution,
χ, F, R, T have their usual meaning.

For "leaky" membranes (glomerula, capillary wall, intestinal mucosa) a flow term for osmotic drag has to be added which can be influenced by the antidiuretic hormone. Ussing considers all transport obeying the flux equation as passive.

Gel filtration suggests linking of extended macromolecules, either by cross-linking or by a fine fibrous network. Here must be remembered the "spinning" properties of mucoproteid matrices, which will set up barriers to macromolecules and prevent a mass-movement like spreading. Gel filtration can be expected only if the barrier has a certain thickness (aortic membranes, dermal membranes), so it has no interest for axon membranes and for intracellular structures.

For these very thin membranes HUTCHINSON's survey of radiation effects on monolayers is important [4]. Monolayers, or very thin barriers, may influence radiation effects in two ways: they cause orientation and polarization of large molecules, and of ions, and they make cages and channels for intracellular organization. It is evident that freeing of enzymes, liberation of co-enzymes and, for example, of neurohormones, must cause a large and varied amplification of the primary radiation absorption effect. At present no direct evidence of radiation rupture of cellular membranes is available, although indirect indications are increasing. Radiochemically, one hypothesis goes in the direction of the formation of lipoperoxides, with the possibility of autocatalytic chain reactions. But the reported high yields here [5] have not been confirmed. The oxygen effect could also be brought into play. Another hypothesis suggests a liberation of neurohormones by "stress", followed by rapid attack of, for example, acetylcholine on the axonal or synaptic membranes. The specificity and intensity of this reaction could be well understood, if it is supposed that cholinergic enzymes are a structural part of the membrane. I owe the latter idea to my colleague, Prof. Dr. J. A. Cohen. The histochemical localization of cholinesterase, and especially of choline acetylase, supports it, and here also autocatalysis may be very important: it appears as a result of the compartmentation of acetylcholine in vesicles [6].

As to the subject of this meeting, two membrane properties emerge; namely, the influence of radiation on polarization and on the liberation of neurohormones. Regarding the first, I can only suggest that a radiation "hole" in a functional membrane, in equilibrium with cytoplasma, might exist for not more than microseconds,

and then be filled up again. This could be long enough for depolarization to begin, but it would not permit the leaking of enzymes, and so on; for this, secondary processes must occur.

The above considerations chiefly hold for "passive" impermeability and location. It is evident that, superimposed upon this, an important active impermeability, with carriers and cycles requiring energy transformation, comes into account. Examples are, the sodium pump at the surface of many cells, the concentration of neurohormones against a steep gradient in thrombocytes or in synaptic vesicles, with the aid of ATP, the reabsorbtion of glucose and of amino acids in renal tubuli, etc. It is probable that X-ray interference with these "pumping" processes is the main cause of liberations and depolarizations. If decrease in ATP synthesis is an early X-ray damage, then a local lack of ATP might affect the concentration mechanism across a cellular barrier and consequently depress the "bound" state. In this way the radiation liberation of ATP-ase [7] might be very damaging.

Another possible point of attack might be the membrane sodium phosphatidate cycle, as described by HOKIN and HOKIN [8].

These authors showed, in brain slices, and particularly in preparations of the salt-excreting gland of the albatross, that acetylcholine stimulates the turnover in the synthesis and hydrolysis of phosphatidic acid by the enzymes diglyceride kinase and phosphatidic phosphatase. Phosphatidic acid is especially suited to the formation of sodium phosphatidate, which is soluble in the fatty part of cellular membranes, and thus may act as a sodium carrier. Hokin's scheme of active sodium transport is given in Fig. 1.

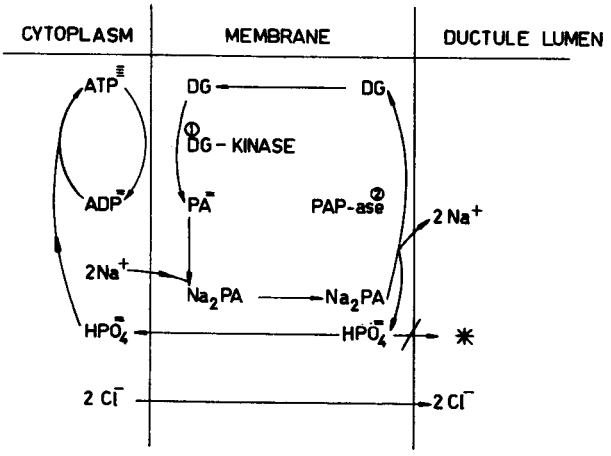

Fig. 1

The phosphatidate cycle for sodium transport

Active sodium transport gives rise to an electrical potential difference which provides the force for moving the chloride ions. The mechanism seems to be specific to sodium ions. X-ray influence might act on the unsaturated fatty acids

of phosphatidic acid in the presence of oxygen; another reaction might be set up in the protein binding of the phosphatidate. (The effect of a decrease in ATP synthesis has already been mentioned.)

Turning now to axonal and synaptic membranes, it has been found that X-rays influence ionic permeability, with consequent alterations in polarization, but only at high doses. In the giant axon of the squid, ROTHENBERG [9] showed that, immediately following exposures to 50000 and 12500 r, a marked increase in Na permeability across the axonal membrane was evident. BACHOFER [10] found that irradiation at a dose of 6 kr/min caused a considerable increase in spike potentials in the caudal nerves of rats on excitation by square wave single supramaximal stimuli; this might be explained by the increased ionic permeability described by Rothenberg.

For low-level irradiation effects in the central nervous system, which very probably originate from membrane alterations, I quote part of the summary made by BACQ and ALEXANDER of the monograph by LEBEDINSKY, GRIGORYEV and DEMIRCHOGLYAN [11]:

"(a) Changes in the threshold of electric stimulation of the cat's hypothalamus after 50 r of total body irradiation.

(b) Depression of cortical activity in man, preceded by a short phase of increased activity after head irradiation.

(c) A rise in cortical excitability of the rabbit 10 s after beginning of a total body irradiation at 0.13 r/s; depression was seen 1 h after a dose of 7—8 r."

There are many other papers which could be mentioned, but they are not relevant here.

I wish to stress only one more point which appears somewhat neglected at present, and that is, the influence of an optimal Ca ion concentration. This is important in any membrane permeability problem; the large influence on hydrolysis of ATP as studied in mouse spleen homogenates, especially after irradiation, is interesting for radiobiology [12]. The optimal Ca^{++} concentration appears here to be 1 mM/l; with more, or less, Ca^{++} ions, hydrolysis is much decreased.

The increased irritability of nerves at low Ca^{++} is well known; a fresh sciatic nerve of the rat exhibits spontaneous repetitive discharge, amounting to some 100 μV, if Ca^{++} is too low in the bathing solution [13]. The stabilizing action of Ca^{++} might arise in some cases from lipid phosphate linking or, in others, from mucosulphate linking.

In general, an optimal Ca is necessary for normal permeability of cell surfaces and nuclear membranes; on the other hand, for the experimental collection of mitochondria, Ca^{++} must be very low if disintegration is to be prevented.

With the influence of irradiation on the liberation of neurohormones, we enter a rapidly expanding field, so vast that only a few instances can be mentioned. We are not very certain what the state of binding may always be, but it appears certain that "vesicles", especially synaptic vesicles, are preponderant. One fact to be kept in mind is that "vesicles", like thrombocytes and mast-cells, often contain not one neurohormone, but several which may be antagonistic [14]. So "liberation" almost always results in the increase of several "free" neurohormones, which are then no longer protected against their corresponding oxidizing enzymes.

The distribution of mast-cells along the nerve sheaths [15] does complicate the study of direct nerve and synapse irradiation; the possibility of a direct effect on, say, excitability, actually being effected under neurohormonal influence, must be thought of.

It is generally admitted that irradiation, if strong enough, may be considered as a "stress", evoking an alarm reaction exhibiting the usual symptoms; this may hold for total body and for local irradiation. But the primary eliciting process is not known, and it is not very remarkable that a local dose which is sufficient to produce destruction of tissue, will set up an alarm reaction. One is often left with the impression that here again the primary biological event is the liberation of neurohormone combinations by irradiation, the decompartmentation taking place centrally (hypothalamic) or peripherally (intestinal, abdominal organs). The cause of early radiation sickness a few hours after irradiation might also belong here. Some observations are now given from which the theory just mentioned may be deduced. For clarity, they are restricted to very early phenomena.

Gastric retention is a well-known early symptom in animals and in man. It has been shown by LAMBERTS and DIJKEN [16] to be always present in rats 2 h after receiving a dose of 100 r, to be prevented by adrenalectomy, and to be imitated by the deposition of 1 mg serotonine + 1 mg adrenalin in oil. Moreover, the presence of both hormones and their oxidative metabolites can be detected in urine after irradiation. Their increase in the blood has sometimes been detected, but this is complicated by their rapid oxidative destruction.

Another simple, early reaction is the spreading of pigment in the melanophores of a hypophysectomized frog. Here the balance between MSH and epiphyseal melatonin (5 methoxyacetyl-serotonin) is disturbed, leading to centralizations of the pigment in the pale animals. This can be reversed by serotonin, DAVEY [17], and also by X-irradiation with 400 r [18]; the effect is distinct after 10 min. Again the presence of serotonin and metabolites in urine after irradiation is very convincing.

It is interesting to see how the pigment expansion in isolated pieces of frog skin, immersed in salt solution, can be built up by the successive addition of micrograms of neurohormones. In the absence of MSH, melatonin + serotonin + adrenalin + ATP + glucose + oxygen are necessary.

Another suitable preparation for studying serotonin release is the rat's uterus, extirpated during oestrus, and enriched in serotonin by a previous intraperitoneal injection of 5 OH tryptophane. In a quiet preparation, 100 r of X-rays will start a period of rhythmic contractions, characteristic of free serotonin. Changes in blood pressure after irradiation may point in the same direction, but their interpretation is rather complex. There are many more findings, but those mentioned here may suffice. What I wish to say is an extension of the enzyme release theory of BACQ and ALEXANDER, and a similar proposition on the radiation release of neurohormones. Autocatalytic phases may occur in neurohormone liberation as well as in enzyme release.

The occurrence of histamine liberation by heat, friction, UV, pharmacological liberators and allergic reactions is such a general phenomenon that it is also expected from ionizing radiation. There is ample evidence that after X- or γ-irradiation its blood level rises, the renal excretion increases, and some organs become depleted. BACQ and ALEXANDER should be consulted for a survey [31]. The possible significance of histamine as a neurohormone is not as evident as it is for the amines mentioned earlier.

We will now have to see what evidence can be collected on X-ray-sensitive receptors initiating further nervous activity. It is natural to begin with photoreceptors, and here the excitation of the retina is one of the oldest topics in radiobiology. In 1896 AXENFELD [19] thought that insects and crustacea were attracted by X-rays and that animals without retinae were not. BRANDES (1896) [20] knew

that humans observed a light sensation, a phosphene, but he could not then discriminate between direct excitation of retinal elements and primary fluorescence of occular tissues. Röntgen himself, who at first denied "visibility" of X-irradiation, later confirmed it. In 1902, HIMSTEDT and NAGEL [21], by means of a simple electroretinogram in dark-adapted eyes, localized the effect in the rods. They accepted primary fluorescence.

Thirty years later, PIRIE [22] showed that patients could read short words originating from lead letters forming an X-ray shadow on the retina. Much later fine pencils of X-rays were used for determining the dimension of the eyeball by transverse irradiation. A small transverse bundle is observed as two bright points by the dark-adapted patients.

In 1933 THIER [23] made the first quantitative observations by registering the ERG of an enucleated frog's eye which had been exposed to gamma irradiation from radium. He could exclude fluorescence and found much analogy with stimulation by visible light. Twenty years later the study was resumed by LIPETZ [24], with modern apparatus. Lipetz measured opticus nerve potentials evoked by X-ray retinal stimulation in frogs. He concluded that the effects of visible light and of X-rays are highly analogous. The chief difference is that, with X-rays, recovery time is much longer, although reversibility remains intact during the first half-hour. We confirmed Lipetz's work with the ERG of the dark-adapted frog's eyes, and showed that X-irradiation and light irradiation are additive. Sensitivity to light is decreased by X-rays (a very short increase in sensitivity may precede) and to X-rays by light. The Xenopus eye, which does not possess rods, is not sensitive to X-irradiation.

The sensitivity of the dark-adapted eye to X-rays is not much less than to visual light, if studied with optic nerve spikes or with the ERG. Sensitivity to P^{32} or Sr^{90} in a suitable solution and directly touching the retina, has also been demonstrated. More refined studies have been made by many Russian and Japanese authors; for these, the survey by LEBEDINSKY [11] should be consulted.

A more general, or primitive, receptivity to ionizing radiation may be derived from experiments in which free-moving animals evade low-level X-irradiation. This appears to hold for rats [26], mice [27] and *Daphnia* [28]. The film by HUG, in which snails are seen to retract their feelers at a dose-rate of 1.5—5 r/s, with a latency of 5—15 s, is well-known. Many other lower animals were also seen to react to low doses. HUG [29] discusses his results in the following way: "One is tempted to interpret the reactions of the lower animals as a sort of röntgenphen, evoked in photo-receptors. In all the lower metazoa we have examined, even in the absence of developed eyes, a marked sensitivity to light is evident. It is not, however, under exposure to light or an increase in light intensity that all these animals show reflex actions similar to those they show under X-rays, but under a decrease (shadow reflex). Furthermore, some reactions observed under irradiation may hardly be explained by stimulation of photoreceptors; for example, the typical defence reactions of ants, which point to a chemical effect on the organs of taste or smell, or the preliminary studies on the muscle-nerve preparations of worms".

It will not be easy to differentiate the numerous "radiation reflexes" on lower metazoa from effects set up by the liberation of biological amines; perhaps the combination of both processes will best explain the observations.

The occurrence of vesicles containing neurohormones in visual and in taste receptors has been described by DE ROBERTIS [30]. Their possible significance in radiation sensitivity has already been discussed.

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DISCUSSION

D. Rosen (United Kingdom): With regard to Dr. Brinkman's paper, one should realize that the study of neurohormones released after X-irradiation of an intact animal, or an isolated organ, is complicated by the possibility of physiologically indirect effects. I would like to mention some experiments carried out in London by Dr. Alexander, of the Chester Beatty Research Institute, and Dr. Mongar,

of University College. They irradiated minced guinea-pig lung with doses of up to 7 kr. Although this tissue readily releases histamine *in vitro* under suitable conditions, X-irradiation produced no detectable histamine release. However, there were indications that irradiation increased the strength of response to anaphylactic shock. If a guinea-pig had been sensitized with egg albumin, histamine was released *in vitro* from minced lung on addition of the antigen. *In vitro* irradiation of the minced lung tissue (with doses of a few thousand roentgens) appeared to increase the amount of histamine released by the antigen. Of course, I do not deny that, in the intact, irradiated animal, evidence can be found of histamine release; but the release may be entirely a secondary effect.

R. Brinkman (Netherlands): I agree there is a gap between the apparent sensitivity of "bound" neurohormones in living cells and the great resistance to liberation in vesicles or granules, in isolated dispersion. This undoubtedly presents a problem.

A. V. Lebedinsky (Union of Soviet Socialist Republics): Dr. Brinkman's interesting report presents a particular point of view in a very persuasive manner. I would like to ask one specific question: when we use X-rays on a frog's skin and obtain, as a response, a difference in potential on the skin, this may be due to the freeing of a neurohormone, but I should expect this to be the case only where the same effect would have been obtained after section of the sympathetic nerve of the skin, because we often obtain such variations in skin potential under the influence of chemical or other excitation. In other words, it is important to find out what part the sympathetic effect plays in regard to the skin glands of the frog, which are prone to stimulation by reflex action, as a result of excitation of the skin receptors. I should also like to add that your point of view helps us to understand why we obtain so clearly the darkening effects in irradiation of the whole frog and never see these effects on an isolated segment of skin.

R. Brinkman: I mentioned this experiment on the expansion of melanophores, or of the melanophore pigment, as an example of how neurohormones play an intermediate role. However, I certainly would not exclude the sympathetic system, though there, too, the final action is the liberation of noradrenaline, so the neurohormone is involved.

With a view to determining how far you have to irradiate the frog, and whether you can have expansion of pigment in other places, you can do many good experiments. You can irradiate one place and get an expansion on many other places after a short time. It looks, therefore, as if it is quite the same as the well-known psychogalvanic reflex, which is certainly mediated by the sympathetic system. Furthermore, if you can divide the frog into a front and a back section, connected only by the sympathetic chain, you still have this reaction. Hence I think there is no difference between your opinion and mine. In the last analysis it is a sympathetic stimulation of the glands in the skin of the frog, and that is mediated by some neurohormone.

T. J. Haley (United States of America): Histamine is liberated by X-irradiation in doses of 600, 900 and 1200 r in the rat. If the histamine liberator, 48/80, is given before or after the radiation, the amount of histamine liberated is the same. If diamino oxidase is inhibited by aminoguanidine, the amount of histamine liberated increases by a factor of 3. Furthermore, chronic administration of 48/80 for 20 d does not completely deplete the histamine stores because X-rays will still liberate histamine. (LEITCH *et al.*, *Amer. J. Physiol.* **187** [1956] 307.)

The decreased gastric emptying time produced by X-irradiation in the rat is due to pylorospasm. One should recall that the pylorus goes into spasm when the sympathetic nerves to the stomach are stimulated; thus the neurohormone, noradrenaline, is involved. (DETRICK *et al.*, *Amer. J. Physiol.* **179** [1954] 462.)

R. Brinkman: With regard to the second remark, I think that gastric retention is not caused by pylorospasm. By fluoroscopic observation we saw no evidence of pylorospasm, but we did see a distinct inhibition of gastric movement. In adrenalectomized rats given a "dépôt" of adrenalin in oil, we found no gastric retention, only the combination of adrenalin and X-irradiation results in the retention found in normal irradiated animals.

J. Nicholls (United Kingdom): Is there any direct evidence for your statement that synaptic vesicles in the region of the neuromuscular junction are vulnerable to irradiation? Our finding that miniature end-plate potentials are unaffected by large doses would suggest that this is not the case at this site.

R. Brinkman: I must agree again, as in the answer to Dr. Rosen, that there is a gap between apparent sensitivity in certain experiments and resistance in more direct observations.

W. R. Stahl (United States of America): The time course of neurohormone release from neurovesicles presents puzzling problems. If it is immediate, there should be a brief shower of hormone release having a transitory effect. If the vesicle rupture occurs late, then it must be presumed to be an indirect radiation effect, due to local tissue injury. In either case the correlation with possible behavioural effects is hard to understand. Do you think that the local release of neurohormones can be of adaptive value to radiation-affected cells that will presumably die because of injury to normal mitotic mechanisms?

R. Brinkman: It may be pointed out that released neurohormones are generally good chemoprotectors against irradiation; serotonin, in particular, is one of the best protectors. But there is still scope for much research on this whole question.

O. Hug (Chairman) (Federal Republic of Germany): The following experiments, which were performed in accordance with Professor Brinkman's advice, could be considered as supporting the hypothesis of release of neurohormones by radiation. The frog lung is suitable for testing serotonin. If the frog lung is suspended on the lever of a kymograph rinsed with Ringer's solution and irradiated with 50 kV X-rays, the lung relaxes immediately under irradiation and recovers in the course of minutes. Sometimes the relaxation is followed by contraction. If the preparation is in a labile state, i. e. shows spontaneous rhythmical movements, the radiation can induce short-lived additional movements of the lung. The lowest dose after which changes of mobility tonus could be observed was about 250 r.

With regard to photo-receptors, I remember, Prof. Brinkman, that at one point you showed that after repeated pulses of X-rays there was a very quick fatigue. But I think this was not observed in the case you have described. Is that correct?

R. Brinkman: Yes, if you have stimulations of 1/min you have no fatigue, but if you go a little below that you have a clear fatigue phenomenon. I think that is what Dr. Bachofer also found in much better preparations. There certainly is a fatigability which is greater than that for light.

O. Hug (Chairman): I think this fact cannot be explained merely by the effect on the visual purple and indicates that nervous elements of the eye are also affected by irradiation.