level within the complex, yielding the reactive triplet state of B by dissociation.

From a thermodynamic point of view it is certainly convenient to employ a steady state constant related to the constant of quenching, as an alternative to the more conventional use of collisional cross sections[36,37]. The transfer of excitation energy between different energy levels of the system is then capable of clearer definition[38]. In particular, the probability term P in the diffusion-encounter treatment may be correlated with transitions between available excited levels in A and B[21]. Finally, the concept provides an interesting analogy between photoexcited systems and enzyme systems where the hypothesis of the steady state 'MICHAELIS complex'[39] has proved so fruitful[40,41].

Zusammenfassung. Die Kinetik homogener Photoreaktionen, welche über einen Excimer-Komplex verlaufen, wird untersucht. Es ergibt sich, dass in Übereinstimmung mit zahlreichen Beobachtungen eine Konzentrationsabhängigkeit ähnlich der von LANGMUIR-HINSHELWOOD für die heterogene Katalyse abgeleiteten zu erwarten ist.

J. H. TURNBULL

Applied Chemistry Branch, Department of Chemistry and Metallurgy, Royal Military College of Science, Shrivenham (Berks., England), 2 August 1967.

[36] K. J. LAIDLER, J. chem. Phys. *10*, 42 (1942).
[37] R. G. W. NORRISH and W. SMITH, Proc. R. Soc. A *176*, 295 (1940).
[38] J. FRANCK and H. LEVI, Z. phys. Chem. B *27*, 409 (1935).
[39] G. E. BRIGGS and J. B. S. HALDANE, Biochem J. *79*, 383 (1925).
[40] J. H. TURNBULL, Experientia *20*, 113 (1964).
[41] I am indebted to Dr. E. J. BOWEN, and to a referee of Experientia for helpful criticism.

Combined Action of Rous Sarcoma Virus and Chemical Carcinogen in Rats

The combined effect of oncogenic viruses and chemical carcinogens has been extensively studied in recent years (for review see DURAN-REYNALS[1], and SALAMAN and ROW[2]). The possibilities with the combined action can be summarized as: (1) enhancement of the oncogenic effect of the virus; (2) enhanced oncogenic effect of the chemical carcinogen; (3) simple additive effect of the 2 agents.

The first alternative was most often observed: viral tumours appeared earlier and/or in greater number, grew more rapidly or persisted for a longer time in carcinogen-treated animals than in the controls which had only been treated with virus. Enhanced effect of the chemical carcinogen by virus has rarely been observed.

Few investigations have been carried out on the effect of Rous sarcoma virus (RSV) in animals treated with chemical carcinogens. CARR[3] injected methylcholanthrene and RSV into chickens belonging to a strain of low viral susceptibility. Small tumours appeared in the breast muscles at the site of virus inoculation, whereas a large swelling developed in the methylcholanthrene-injected leg. The swelling slowly subsided and tumours subsequently appeared in various parts of the leg, which were histologically indistinguishable from those induced by the RSV. In young rabbits i.v. RSV has been shown to localize to the site of i.m. injected hydrocarbons, producing fibromatous nodules[4]. In addition many rabbits showed nodules in the lungs and liver, occasionally also in the spleen and kidneys. The nodules were larger and more numerous than in rabbits not treated with hydrocarbon.

The present experiments were carried out to investigate the effect of i.v. RSV in rats, which had been injected i.m. with a carcinogenic hydrocarbon.

The Rous virus was of the strain Schmidt-Ruppin (RSV-SR) which is able to induce tumours in a wide variety of mammals as well as in birds[5]. Pools of cell-free virus suspension were prepared from rapidly growing tumours induced in the chicken. To do this finely minced chicken sarcoma was suspended 1:5 in Hank's solution with antibiotics and homogenized for 5 min in an Ultra-thurrax homogenizer (24,000 rpm) in the cold; the suspension was then centrifuged for 30 min at 3000 *g*. The supernatant was pipetted off and stored at − 70 °C. Two pools were used in the experiments. The titer of the virus was $1.5–2.4 \times 10^6$ FFU/ml tested on monolayers of chick fibroblasts. The rats were white ones, kept as a closed colony for many years at the institute. 7,12-Dimethylbenz(*a*)anthracene (DMBA) dissolved in arachis oil or trioctanoine was used as a carcinogen.

One mg DMBA was injected i.m. into the left thigh of 41 rats, 2 weeks of age. 10–12 days later, 28 of the rats were given 1 ml of the virus pool i.v. via one of the tail veins. The same amount of virus was injected into 16 rats of the same age which had not been treated with the carcinogen. The rats were examined once a week for 4 months and then killed.

No tumours were observed in any of the rats which had been given the virus alone, nor did they show any hemorrhagic cysts in the lymph nodes which is a common finding when RSV-SR is inoculated into new-born rats.

Seven of the 13 rats which had been injected with DMBA and had not had any further treatment, developed a tumour at the site of injection. The first tumour appeared approximately 12 weeks after the injection and had often reached a considerable size by the time the rats were sacrificed. A few of the tumours had produced ulceration of the overlying skin. They had the histological appearance of various types of sarcomas: spindle cell sarcomas, polymorphous cell sarcomas, myosarcomas and anaplastic sarcomas. No metastases were seen in any of the internal organs or in the lymph nodes.

Fifteen of the 28 rats that had been exposed to the combined effect of DMBA and RSV-SR developed tumours in the left thigh at the site of the injected hydrocarbon. The tumours appeared at about the same time as in the rats treated with DMBA alone and were of the

[1] M. L. DURAN-REYNALS, Progr. exp. Tumour Res. *3*, 148 (1963).
[2] M. H. SALAMAN and F. J. C. ROE, Br. med. Bull. *20*, 139 (1964).
[3] J. G. CARR, Br. J. exp. Path. *23*, 221 (1942).
[4] C. G. AHLSTRÖM and J. MARK, Int. J. Cancer *1*, 51 (1966).
[5] C. G. AHLSTRÖM, S. BERGMAN, N. FORSBY and N. JONSSON, Acta Un. int. Cancr. *19*, 294 (1963).

same histological type. Eleven of the 28 rats developed tumours at distant sites which had no topographical relation to the injected hydrocarbon. These tumours were localized to the liver (4 rats), to the cheek invading the orbit (3 rats), to the base of the right ear (1 rat), to the back of the neck (1 rat), to the right side of the spine (1 rat) and to the abdominal skin (1 rat). Three of these tumours appeared in rats which had not developed a tumour at the site of injected hydrocarbon. One rat showed a 1 × 1 cm sized tumour attached to the spine and a small tumour nodule in the liver. Another had a liver tumour 2 × 2 × 3 cm in size and in addition numerous tumour nodules in the omentum, on the peritoneum and a 0.5 cm tumour nodule attached to the upper surface of the diaphragm. The tumours had a hard consistency and grayish-white cut surface. Microscopically they all had the appearance of fibrosarcomas, the majority highly differentiated.

The tumour in the abdominal skin appeared 9 weeks after the virus inoculation, those on the head and neck after 12 weeks. They grew slowly but progressively. The liver tumours were detected at autopsy.

Two of the rats showed in addition to the tumours, thin-walled cysts filled with hemorrhagic fluid. One of them had a thymic cyst and 2 × 2 × 2 cm sized tumour on the back of the neck, the other had apart from a liver tumour, numerous tiny hemorrhagic cysts in the lungs, a hemorrhagic effusion in both pleural cavities and numerous small cysts in the abdominal cavity. Three of the rats showed hemangioma-like cysts in the lungs and spleen or on the peritoneum, apart from the sarcoma at the site of the hydrocarbon.

A schematic summing up of the results of the experiments is given in the Figure.

DMBA given orally to young rats has been shown to induce tumours at various sites[6]. It might therefore be argued that the generalized tumours in our experiments are due only to DMBA and that the injected RSV is of no importance.

The following facts are against this assumption.

(1) No distant tumours were seen in rats injected with DMBA alone.

(2) Some rats with distant tumours showed hemorrhagic cysts which is a common finding in rats inoculated with RSV-SR when new-born.

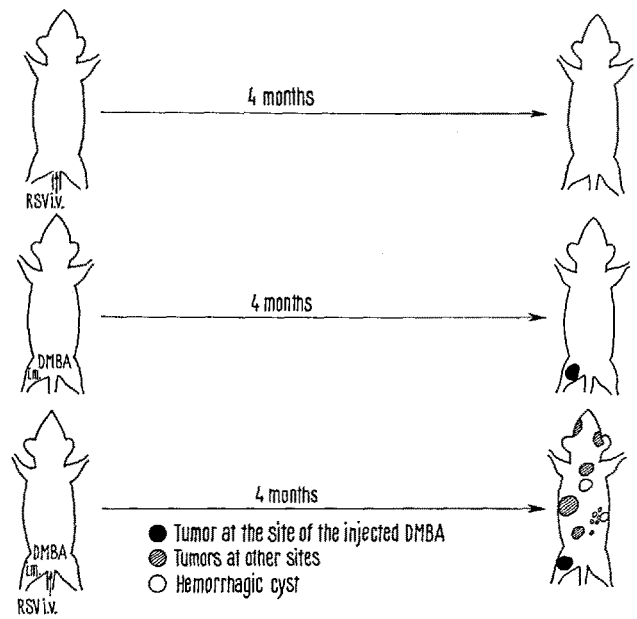
(3) The distant tumours had the same microscopical structure as the fibrosarcomas which appear at the site of injection of RSV-SR in new-born rats. The picture was almost identical in the different rats irrespective of the localization of the tumours and differed from the varied histological structure of the DMBA-induced sarcomas. It should be emphasized that this also applies to the liver tumours which otherwise might be interpreted as metastases from the DMBA-tumour.

(4) In addition, the RSV-SR could be recovered from the liver sarcoma in 1 of the rats. Minced material from the tumour was transferred s.c. to 6 new-born rats and produced rapidly growing tumours in every animal. One of the tumours was finely minced and suspended 1:5 in Hank's solution with antibiotics. One cm^3 of the suspension was injected into the breast muscle of 3 chickens, 3 weeks of age. Two weeks later, 2 of the chickens showed several progressively growing tumours at the site of the inoculation. The tumours showed the usual picture of a slimy Rous sarcoma and could be transmitted to new chickens by means of cell-free supernatant from homogenized tumour material. Earlier attempts to demonstrate the presence of the virus genome in 8 other generalized rat tumours had failed. Tumour material was in these cases transferred directly to the wing webs of young chicks, which were observed for 3 months. It is possible that the virus content is low in very fibrous Rous rat sarcomas, as in slowly growing Rous sarcomas in chickens, and that it increases when the transferred rat tumour cells rapidly proliferate in new-born rats.

The immuno-depressive effect of chemical carcinogens is well documented[7] and it is quite conceivable that the tumours at distant sites in the DMBA-RSV rats are due to a reduced immune reaction to the virus or to an antigen in the neoplastic cells. A similar effect might be produced by other immuno-depressive agents. We have, however, not been able to reproduce the results in cortisonized rats: no tumours or cysts were seen in 15 rats injected at 3 weeks of age with 2.5 mg hydrocortisone s.c. and with 1 ml RSV-SR pool i.v. No results have so far been obtained in rats injected with non-carcinogenic hydrocarbons.

Another possible mechanism is that the chemical agent acts on the target cell, enhancing the effect of the virus or facilitating the entry of the virus into the cell. A synergistic mutagenic effect of RSV-SR and cytidine-triphosphate on human leucocytes has been demonstrated[8]. DMBA has been shown to decrease interferon-production and to enhance the cytopathic effect of polyoma-virus in tissue cultures[9].

It is of interest to note that the tumours showed a long period of latency, in some rats as long or even longer than the DMBA-induced tumours. No satisfying explanation can be given as to their distribution. We have not been able to demonstrate localization of the virus to the



Schematic diagram showing the occurrence of tumours and cysts in rats receiving i.v. RSV (upper group), i.m. DMBA (middle group), or a combination of the 2 (lower group).

[6] M. GRÜNSTEIN, D. R. MERANGE and M. B. SHIMKIN, Cancer Res. *27*, 205 (1967).
[7] J. STJERNSWÄRD, J. natn Cancer Inst. *35*, 885 (1965).
[8] W. NICHOLS, A. LEVAN, W. A. HENEEN and M. PELUSE, Hereditas *54*, 213 (1965).
[9] E. DE MAEYER and J. DE MAEYER-GUIGNARD, Virology *20*, 536 (1963).

site of the injected DMBA as was the case in rabbits and chickens. Transfer of living cells from the DMBA-tumours into chickens did not elicit any tumours. Experiments underway indicate that i.m. injection of RSV-SR into DMBA-treated rats, 4 weeks of age, induces tumours at the site of the virus injection which do not occur in untreated rats[10].

Zusammenfassung. Zwei bis drei Wochen alte Ratten wurden einer kombinierten Einwirkung von 7,12-Dimethylbenz(*a*)anthrazen und Rous-Sarkoma-Virus ausgesetzt. Das chemische Karzinogen wurde i.m., das Virus dagegen i.v., zugeführt. Viele der Ratten entwickelten 3–4 Monate später Fibrosarkomen und Zysten ohne topographische Beziehung zu dem lokal deponierten Karzinogen. In einem Fibrosarkom der Leber konnte der Rous-Sarkoma-Virus nachgewiesen werden. Virus allein war nicht imstande, Tumore und Zysten in gleichaltrigen Ratten hervorzurufen. Das chemische Karzinogen erzeugte Tumore nur am Platze der i.m. Injektion.

C. G. AHLSTRÖM and JULIET HEATON

Institute of Pathology, University of Lund (Sweden), 27 November 1967.

[10] Supported by grant No. CA 06415 from N.I.H. and by the Swedish Cancer Society.

PRO EXPERIMENTIS

A Method for Cytogenetic Study of Planarians

Investigation of chromosomes of planarians in mitosis and meiosis is of particular importance for the knowledge of the problem of polyploidy and its mechanism in animals. Up to the present, for the analysis of chromosomes, squash methods with dissolvable cover slips and acetic-orcein have been applied on gonads and cocoons by MELANDER[1–3]. BENAZZI[4] has combined the acetic-carmine squash method with colchicine as medium on the blastema.

We have modified the acetic-orcein method by pretreatment used in the culture of mammalian cells in vitro. By applying colcemide and hypotonicity it is possible to analyse the chromosomes of sexually mature animals more precisely. The modified method also facilitates the study of the mechanism of meiosis.

Methods. Two species of the endemic genus *Neodendrocoelum* Stank[5], *N. grande* and *N. maculatum*, were taken out of the well of the Lake of Ohrid. The tissues for chromosome analysis were the testes and ovaries of sexually mature animals. The neoblasts of the regenerative blastema in planarians show regional differences in chromosome sets[6]; this somatic tissue was not taken for analysis.

For the pretreatment, colcemide Ciba was employed. At the suggestion of Dr. Y. MELANDER low concentrations of colcemide was used in order to prevent the appearance of induced polyploidization. The concentration of 12 γ/ml gave the best results. This concentration was strong enough to give a contraction effect on the chromosomes but did not produce polyploidy. Colcemide was dissolved in well water in which the animals could survive the pretreatment. Two planarians at a time were placed in a Petri-dish containing 16 cm^3 of colcemide solution, and then transferred into a thermostat at a temperature of 15–16 °C. The planarians were kept in a thermostat for 4–5 h.

In consideration of the low osmotic pressure of the planarian tissues[7], distilled water was used for the hypotonic pretreatment. From the colcemide solution, a planarian was placed on a slide. Under a microscope (objective $\times$ 3) 2 lateral pieces from the testis and the ovary approximately 1 mm in length were dissected using a microscalpel. The 2 dissected pieces of gonads were put into a Petri-dish with distilled water and incubated at 20 °C for 30 min. It should be pointed out that the temperature for hypotonicity was a decisive factor both for the effect of hypotonicity and for the prevention of decomposition of tissues.

After the hypotonic treatment, the pieces of testis and ovary were transferred by a micropipette to 50% acetic acid. Fixation lasted for 4–7 min, and the fixative was changed twice.

The pieces of gonads were transferred, 1 by 1, from the fixative to a slide and macerated with the watchmaker's forceps into a milky cell suspension[8]. A few drops of 2% acetic-orcein in 60% acetic acid were added to the sus-

Table I. Chromosome number of *N. grande*

	Mitosis		No. of animals
	testes	ovaries	
No. of chromosomes	$2n = 32$	$2n = 32$	
No. of cells analysed	12	10	4

Table II. Chromosome number of *N. maculatum*

	Meiosis		No. of animals
	testes	ovaries	
No. of chromosomes	$n = 16$	$n = 16$	
No. of cells analysed	11	21	9

[1] Y. MELANDER, Hereditas *34*, 512 (1948).
[2] Y. MELANDER, Hereditas *36*, 19 (1950).
[3] Y. MELANDER, Hereditas *49*, 119 (1963).
[4] M. BENAZZI, Atti Accad. nac. Lincei, Rc. [8] *40*, 999 (1966).
[5] S. STANKOVIĆ, *The Balkan Lake Ohrid and Its Living World* (Mitgeverij Dr. W. Junk, Haag 1960), p. 357.
[6] M. BENAZZI, Chromosoma *19*, 14 (1966).
[7] P. RÖHLICH, Z. Zellforsch. mikrosk. Anat. *73*, 165 (1966).
[8] T. S. HAUSCHKA and V. V. BRUNST, Hereditas *52*, 345 (1965).