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Review

Systematic Review of Thorotrast Data and Facts: Animal Experiments

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This report represents a *comprehensive survey* of the most important *animal experiments* that have been carried out since the time when the contrast medium thorotrast was first introduced to diagnosis about the year 1930 (Blühbaum et al., 1928; Radt, 1929; Oka, 1929, 1930).

The survey is based upon the study of more than 70 published reports. Although it does not claim to have included *every* publication, it will deal with the most important results and conclusions.

There are *three questions* that have been asked time and again by the investigators:

1. How is thorotrast distributed within the RES?
2. Which and how many tumours can be produced by using thorotrast?
3. Is the tumour-inducing effect of thorotrast caused by radiation, foreign body involvement, or both?

The report is arranged around these questions. Initially, the technical data of the experiments are discussed, then the most important results of these three questions, and finally the resulting conclusions.

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Annotation: Figures 1–12 are photograms of tumours which had developed in rats following injection of different quantities of normal and enriched thorotrast. The animals belong to the experiment of the Working Group "Foreign Body Effect of Thorotrast" which is not finished and whose results have consequently not yet been published. The study is still ongoing.

The Working Group "Foreign Body Effect of Thorotrast" is made up of the following members:
Dr. S. Angermüller (Heidelberg-Ludwigshafen)
Prof. Dr. K. Wegener (Heidelberg-Ludwigshafen)
Dr. H. Wesch (Heidelberg-Ludwigshafen)
Prof. Dr. W. Kemmer (Homburg/Saar)
Prof. Dr. H. Muth (Homburg/Saar)
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Dr. U. Föll (Berlin)
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Prof. Dr. A. Kaul (Berlin)
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Table 1. Range of volumes of thorotrast injected into different animals

Animal	Range of volumes of one injection (ml)	Range of volumes of some injections (ml)
Rat	0.1– 2.0	0.5–56.0
Mouse	0.1– 0.8	
Rabbit	0.3–10.0	ca. 6.0
Dog	2.0–12.0	
Guinea pig	2.0	
Syrian hamster	0.1– 2.5	

In 22 of the 51 animal experiments that have been analysed, rats (the so-called sarcoma animals) were used. 13 times mice, 11 times rabbits, very occasionally guinea pigs, dogs and syrian hamsters.

The number of animals in the experiments varies considerably. Zeitlhofer and Speiser (1954) have observed a single rabbit over a period of 4 years until they noticed the development of a malignant haemangioendothelioma. Roussy et al. (1934, 1936) used, during their important experiments, 20 and later 50 rats. Wenz (1964) has produced the biggest series of experiments so far with 300 rats. Our investigations within the working group "Foreign Body Effect Thorotrast" are being carried out with a total of 2,000 rats (Working Group "Foreign Body Effect of Thorotrast" 1976, 1977). As was the case with human patients, thorotrast was given to animals mainly intravenously (via the tail veins or the ear veins). It was seldom given subcutaneously, intraperitoneally, intramuscularily or submucously.

The injected quantities of thorotrast vary from investigator to investigator. They lie however for the various animals within the following limits (Table 1):

In rats between values of 0.1 to 2.0 ml after one injection and 0.5 to 56.0 ml after several injections; in mice between values of 0.1 to 0.8 ml after one injection; in rabbits between 0.3 and 10 ml after one, and around 6 ml after several injections; in dogs between 2.0 and 12 ml, and in syrian hamsters between 0.1 and 2.6 ml after one injection.

There are only a few investigators (Roussy et al., 1934, 1936; Benstedt, 1963, 1967; Faber, 1973; Wenz, 1964) who worked systematically with several animal groups and with different quantities of injections in order to establish an answer to questions like the following: "can the injected quantity of thorotrast be related to the increasing number of tumours"?, or: "what part does the effect of foreign bodies in thorotrast play in the development of tumours"? In some cases one single animal was given thorotrast repeatedly in regular or irregular intervals. In most experiments the animals were killed after the injection and at a pre-determined time. Only a few experiments were planned as survival studies in the course of which the animals died naturally, either of a tumour or of other causes unrelated to it.

We have planned our unfinished experiment in the working group "Foreign Body Effect of Thorotrast" with some 2,000 rats as a survival study, based on human conditions.

In this way we hope to establish statistically relevant results as to the rôle played by radiation and the rôle played by the influence of foreign bodies during the tumour development (Working Group "Foreign Body Effect of Thorotrast", 1976, 1977). Which answers can be given at this stage to the three questions asked initially?

Distribution of Thorotrast in the RES

Minutes after the intravenous injection, the dextrin – the carrier component of thorotrast – is split off, and this results in a rise of the blood sugar. The thoriumdioxyd particles are surrounded by a protein coating, probably consisting of fibrin (Bell, 1953).

Only then is the thorotrast absorbed by the Kupffer cells by means of pinocytosis and phagocytosis (Fig. 1; Randerath and Schlesinger, 1932). Five min after the injection, thorotrast already appears in the vacuoles of the cytoplasm and is gathered, after some time, in greater agglomerates. These lie over a long period of time in secondary lysosomes (phagosomes) (Tessmer and Chang, 1967; Kluge and Hovig, 1969). The particles which originally measured 5 nm in diameter agglomerated to particles of micrometer size. This agglomeration is concluded after one day (Kaul and Heyder, 1968, 1970).

A short time after the injection, the liver cells also start taking in thoriumdioxyd particles (Fig. 2) but in a lower quantity than do the Kupffer cells (Sampaio, 1956a, b; Grampa, 1965, 1967). Grampa did not find any traces of thorotrast in the gall ducts shortly after the injection.

Hampton and Rosario (1967) saw thorium-dioxyd in the Disse spaces and between hepatocytes 10 min after the injection of thorotrast, and a month later observed dense aggregates in the Kupffer cells of the sinusoides as well as in the liver cells and the gall ducts.

Kabisch (1957, 1967) in experiments with rats determined that not all the Kupffer cells are equally active during phagocytosis, that in fact the number of storing Kupffer cells is related to the injected quantity of thorotrast up to a maximum of approximately 50% of all Kupffer cells. Activation of the RES, for instance by oestrogen, increases the absorption of thorotrast considerably (Grampa, 1967).

In the spleen and the lymph nodes thorotrast is stored more extensively in the red rather than the white pulp (Baillif, 1953; Fig. 3). Repeated injections lead initially to a hypertrophy, later to an exhaustion of the germinal centres, eventually to necroses.

Absorption and storage of thorotrast in liver and spleen is related to the injected quantity and the path of the injection. During this process, the maximum storage in both organs is reached quickly; after that the thorotrast migrates mainly into the lung. The elimination via the kidneys starts after a complete saturation of spleen and liver, only then does thorotrast also occur in the faeces, that is to say, it is eliminated via gall bladder and sputum. The most extensive storage, after greater injected quantities, is observed in macrophages of the spleen and the lymph nodes (Hale and Baillif, 1953).

By using the technique of neutron activating analysis, Kampmann et al. (1968) and Rüger (1969) were able to observe that 9 h after intravenous injection

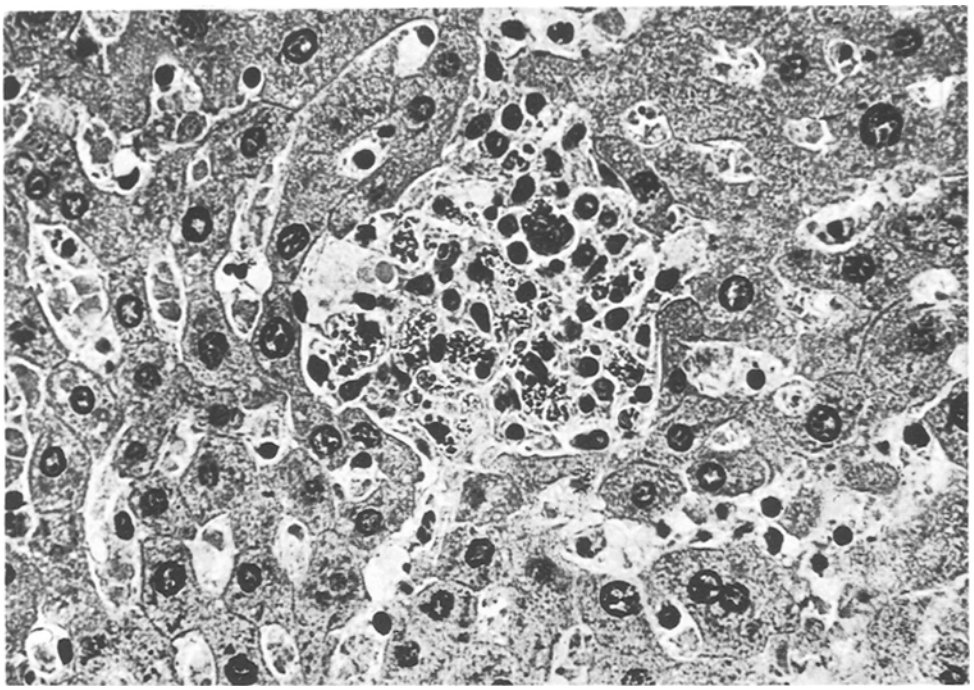


Fig. 1. Thorotrast particles in proliferating Kupffer cells of the rat. (Photogram, HE)

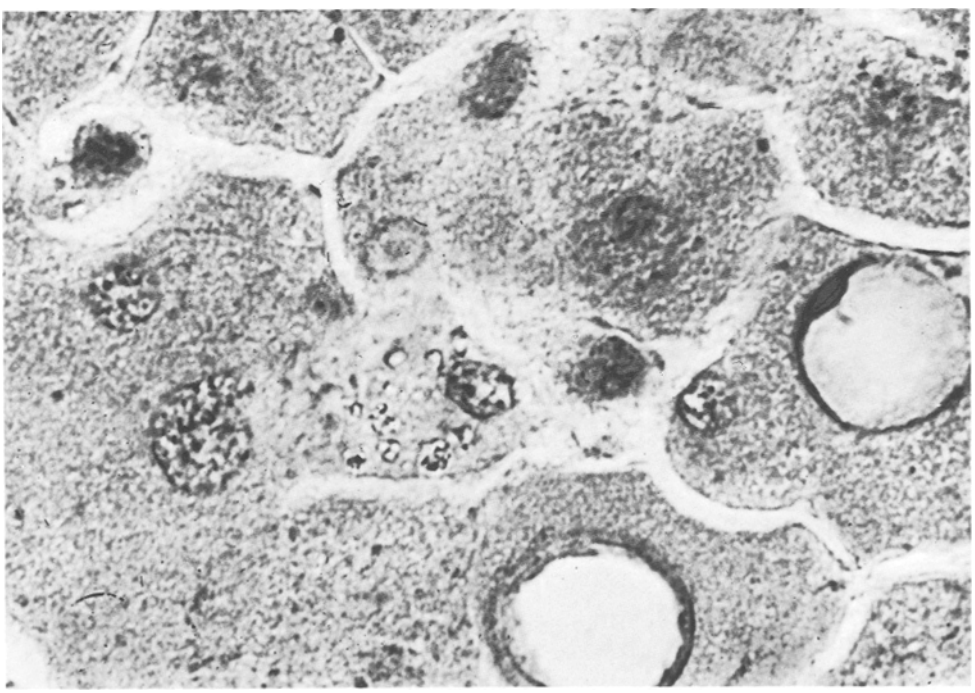


Fig. 2. Thorotrast particles in a liver cell (Photogram; HE)

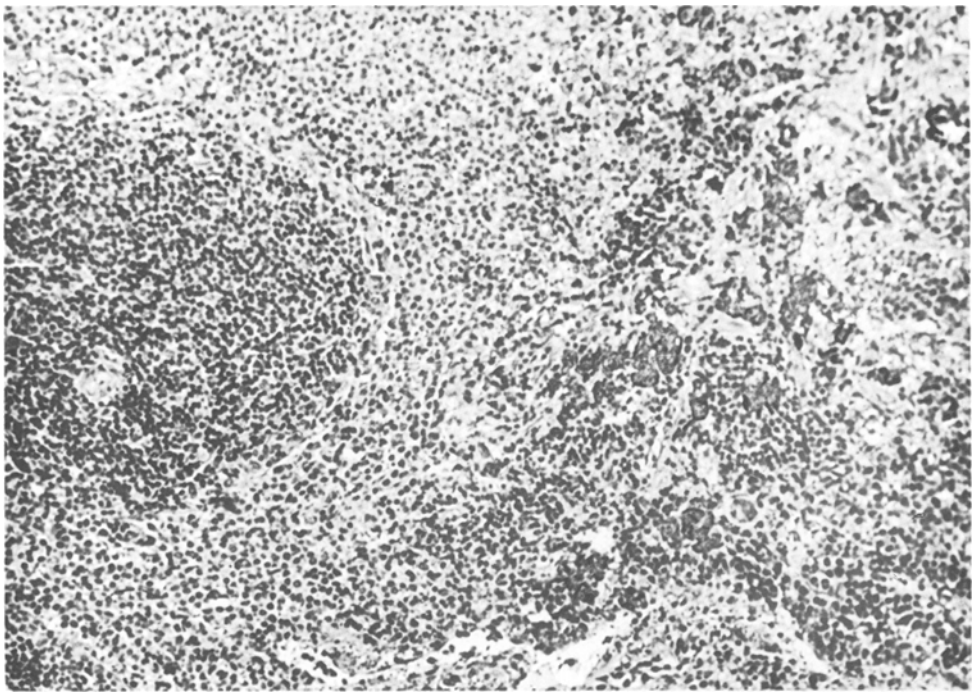


Fig. 3. White pulp of the spleen (rat) with follicle and central artery on the left side. No storage of thorotrast. (Photogram; HE)

into rats the biggest part of the thorotrast is stored in spleen, liver, lymph nodes and suprarenal bodies. Already 3 weeks after intravenous injection, a state of equilibrium in the distribution is reached with approximately 50% of the thorotrast stored in the liver, 20% in the lymph nodes, 5% in the bone marrow and 2 to 2.5% in the spleen.

Results of Tumour Generation (see Table 4)

As early as 1934, that is to say a few years after the introduction of thorotrast into x-ray diagnosis, Roussy et al. (1934) saw fibroplastic sarcomas following intraperitoneal injection of the contrast medium (Fig. 4) and polymorphous sarcomas with extensive atypies of cells and nuclei (Fig. 5).

These results were later confirmed in *rats* by Selbie (1936, 1938), Bogliolo (1937), Mijamoto (1939) and Wenz (1964), in *rabbits* by Johansen (1955, 1960, 1967), in *syrian hamsters* by Mori (1966, 1973), in *guinea pigs* by Foulds (1939) and in *mice* by Selbie (1936, 1938) and Andervont and Shimkin (1940).

Histologically equal tumours in men have been found in the area surrounding thorotrastomas. These tumours can be reproduced in rats by radiation of the scalp using a selected alpha-beam (de Estable-Puig et al., 1970).

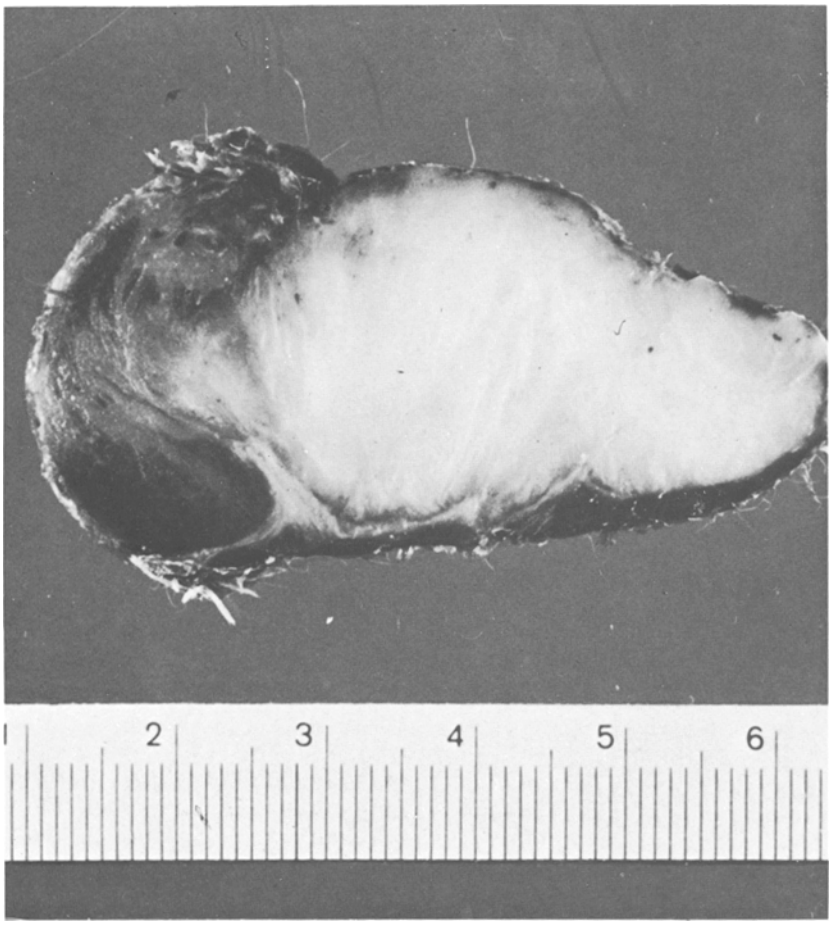


Fig. 4. Fibroplastic sarcoma of the rat, following i.v. injection of thorotrast. (Photogram; HE)

Haemangioendotheliosarcomas in various organs of experimental animals after intravenous injection have been observed much more seldom than in humans. Figure 6 shows such a tumour of the liver in a rat of the experimental series of the working group "Foreign Body Effect Thorotrast". In its histological structure – a varying network of tumour cells with atypies of cells and nuclei – it resembles very much equal tumours in men. This kind of tumour was seen in the liver of *rabbits* by Zeitlhofer and Speiser (1940), in the liver and the spleen of *rabbits* by Swarm et al. (1962), in the spleen, the liver and the lung of *rabbits* by Johansen (1955, 1960, 1967), and in *rats* by Wenz (1964). Haemangiosarcomas also occured after injection of other nuclides, such as Ce-144 Cl_3 for instance (Bayamin et al., 1975).

In some cases, the following benign and malignant tumours were found after injection of thorotrast:

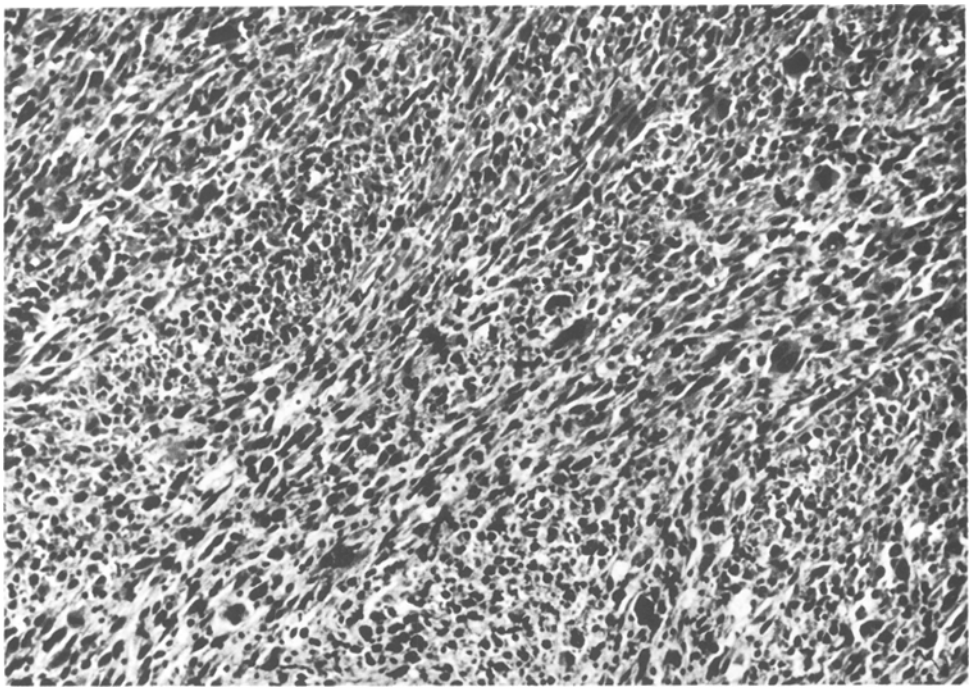


Fig. 5. Polymorphous sarcoma of the rat, following i.v. injection of thorotrast. (Photogram; HE)

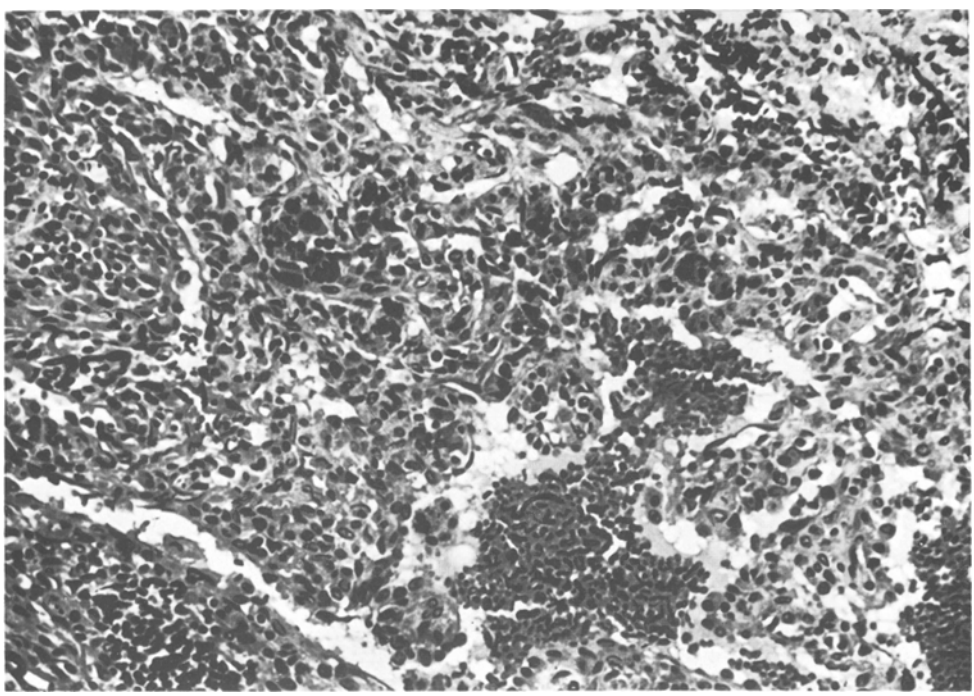


Fig. 6. Haemangioendotheliosarcoma of the liver (rat) following i.v. injection of thorotrast. (Photogram; HE)

1. Focal liver cell hyperplasia (Grampa, Severini, 1963, 1964);
2. Liver cell adenomas (Guimaraes et al., 1955; Guimaraes and Lamerton, 1956; Grampa, Severini, 1963/64) (Fig. 7);
3. Liver cell carcinomas with distinct atypies of cells and nuclei, infiltrating growth and development of metastases (Guimaraes and Lamerton, 1956; Grampa, 1963/64; Wenz, 1964) (Fig. 8);
4. Gall duct hyperplasia (Mori, 1973), recognisable islets of proliferating gall ducts within normal liver tissue (Fig. 9);
5. Cholangiocellular carcinomas (Mori, 1973) with and without metastases (Fig. 10);
6. Malignant haemangioendotheliomas of the spleen that are in accordance with the same kind of tumours in the liver (Guimaraes et al., 1955; Swarm et al., 1962) (Fig. 11);
7. Kupffer cell hyperplasia (Guimaraes et al., 1955; Guimaraes and Lamerton, 1956) (Fig. 12) which could possibly develop into its own kind of sarcoma. In this case it could be identical with a polymorphous liver sarcoma, as was described by Mervenée and Thije (1939).

Apart from this, the following tumours have developed during these experiments and appeared in the published results:

8. Malignant lymphomas (Gilman and Ruckerbauer, 1962);
9. Malignant histiocytomas (Guimaraes et al., 1955; Guimaraes and Lamerton, 1956);
10. Reticulumcell sarcomas (Onufrio, 1938);
11. Osteoid sarcomas (Wenz, 1964);
12. Adenomas of the lung (Guimaraes and Lamerton, 1956);
13. Alveolarcell carcinomas (Johansen, 1955, 1960, 1967);
14. Rhabdomyosarcomas (Johansen, 1955, 1960, 1967).

All these tumours are similar to those known in men. Latency periods vary considerably. Miyamoto (1939) described sarcomas in rats 9 months after i.v. injection of thorotrast. Brunner and Rüttner (1957) on the other hand did not see any tumour even two years after i.p. injection of thorotrast. In our own experiment the shortest latency period of haemangioendotheliosarcoma in the liver of the rat was 15 months.

Which results did the animal experiments reveal with regard to the problem "radiation effect – foreign body influence"?

The researchers of the thirties and forties have blamed the tumour-generating effect of thorotrast almost exclusively on the radioactive radiation of thorium-232. When, in the beginning of the fifties, the sarcogenesis caused by various foreign bodies was very much in the foreground of the discussion (Ott, 1970), the above average occurance of haemangioendotheliosarcomas in the liver and of fibrosarcomas in the area surrounding thorotrastomas was blamed on the foreign body influence of the thorotrast and/or a combination of radiation and foreign body effect.

In 1963 and 1967 Benstedt introduced experiments on various groups of mice that received thorotrast intravenously, Zirkonotrast – a colloidal solution of ZnO_2 with approximately equal particle size as thorotrast – and dextrin (the carrier component of thorotrast). He had hoped to come to a conclusion about the effect of these non-radioactive particles via the group of experimental animals that had been treated with ZnO_2. His results are summarised in Table 2 (column 1 = thorotrast animals; column 2 = zirkonotrast animals; column 3 = dextrin animals; column 4 = controls).

Although the thorotrast animals in the experiment of 1967 have developed the greatest number of tumours of all experimental animals, tumours were

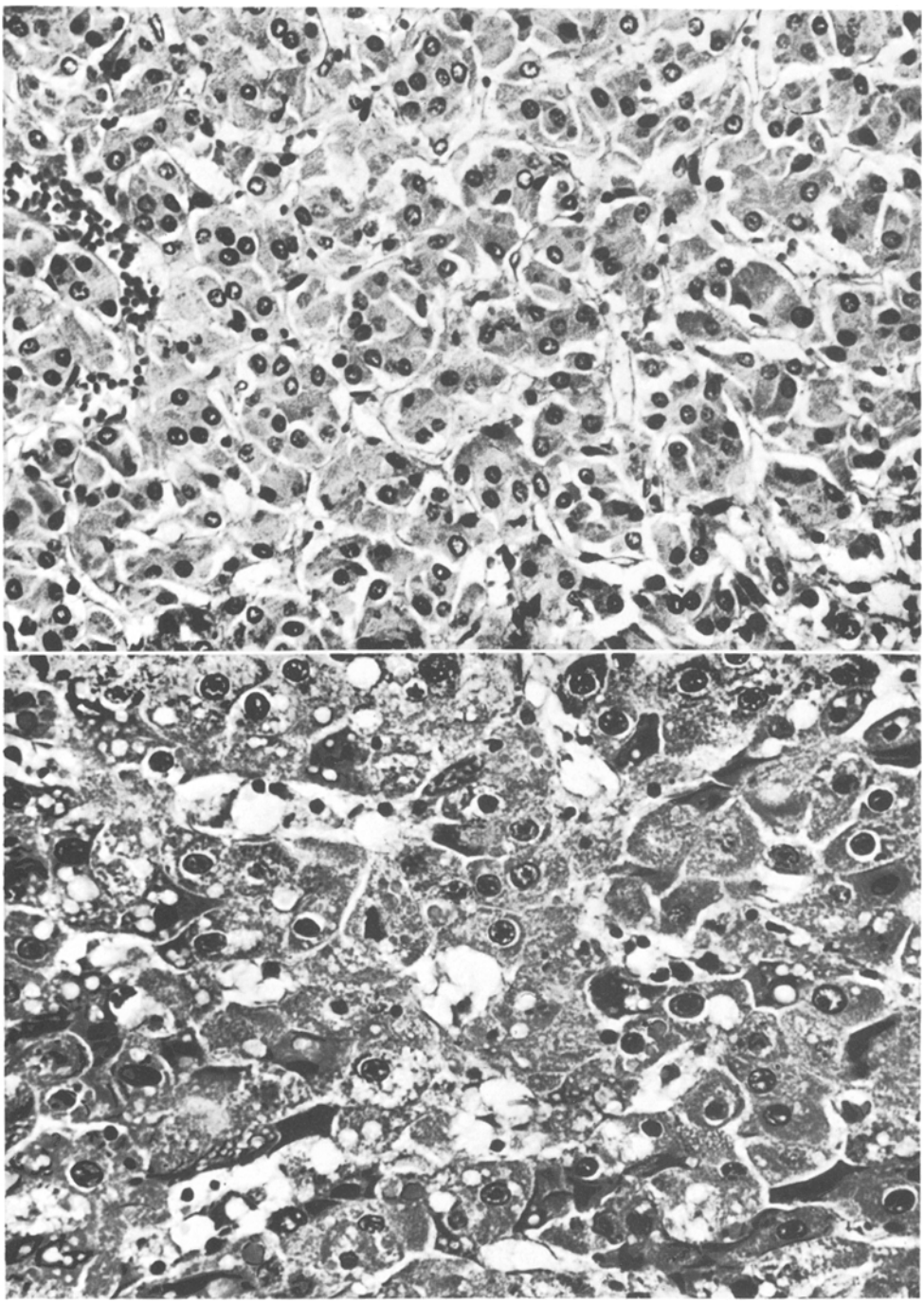
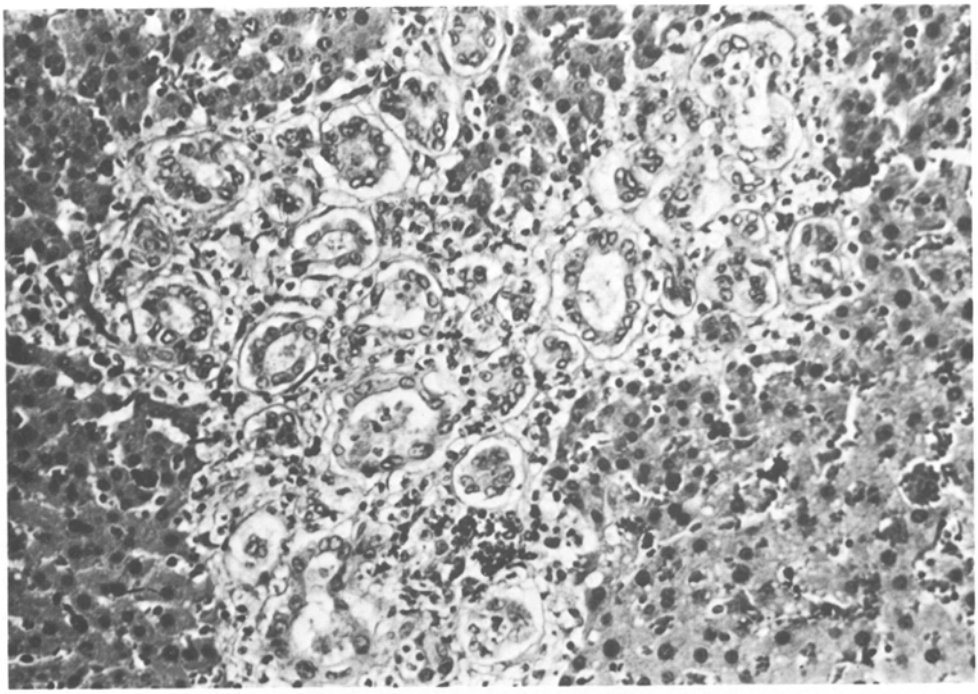


Fig. 7. Liver cell adenoma (rat) following the injection of thorotrast. (Photogram; HE)

Fig. 8. Liver cell carcinoma (rat) following i.v. injection of thorotrast. (Photogram; HE)



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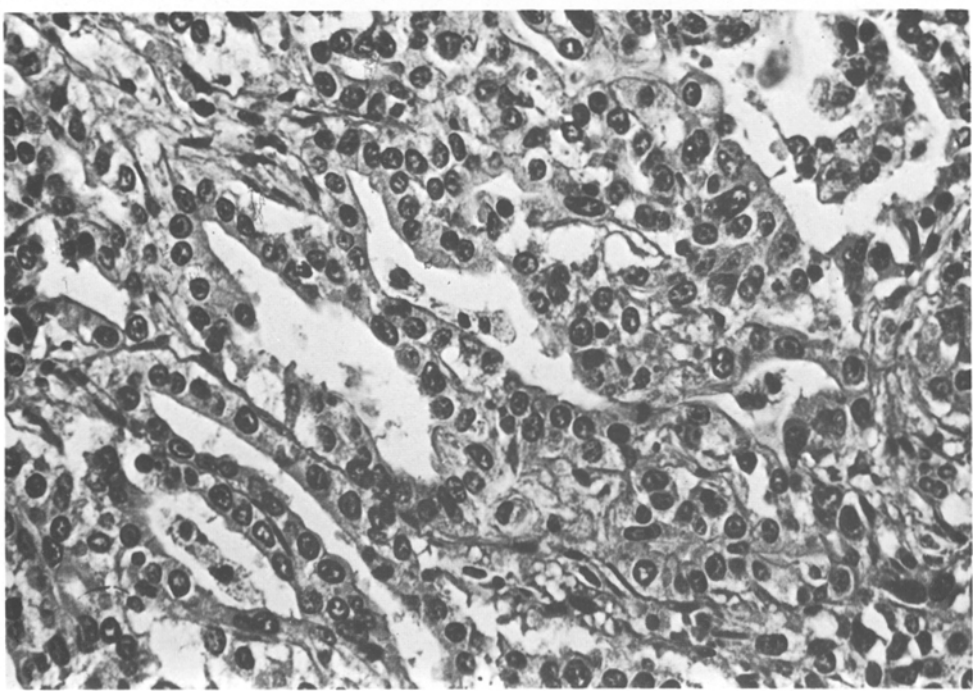

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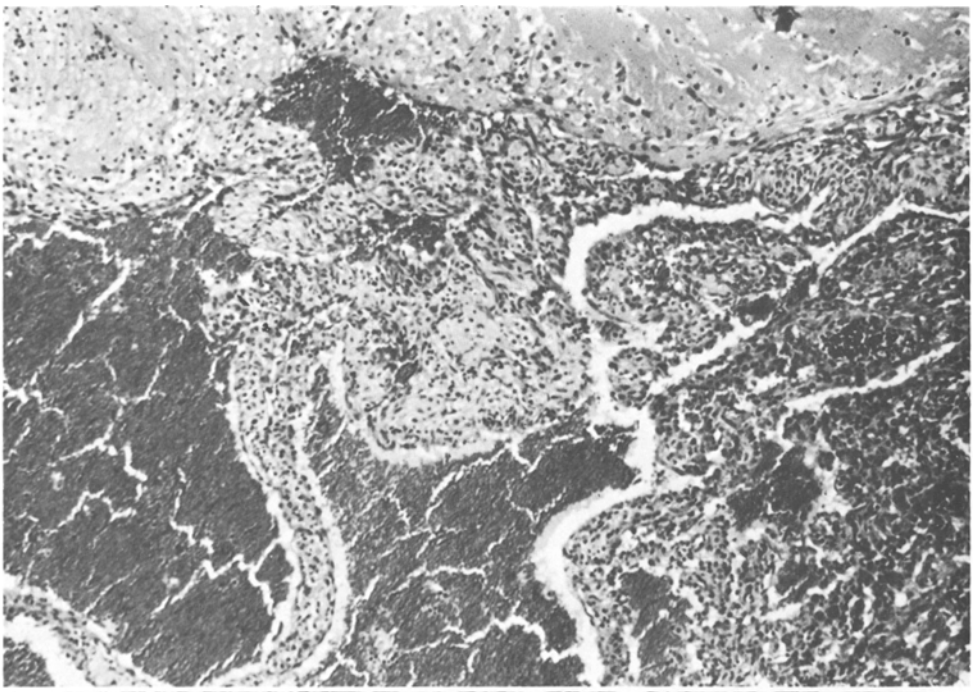
Fig. 9. Gall duct hyperplasia within a portal field (rat) following i.v. injection of thorotrast. (Photogram; HE)

Fig. 10. Cholangiocellular carcinoma in a rat liver following i.v. injection of thorotrast. (Photogram; HE)



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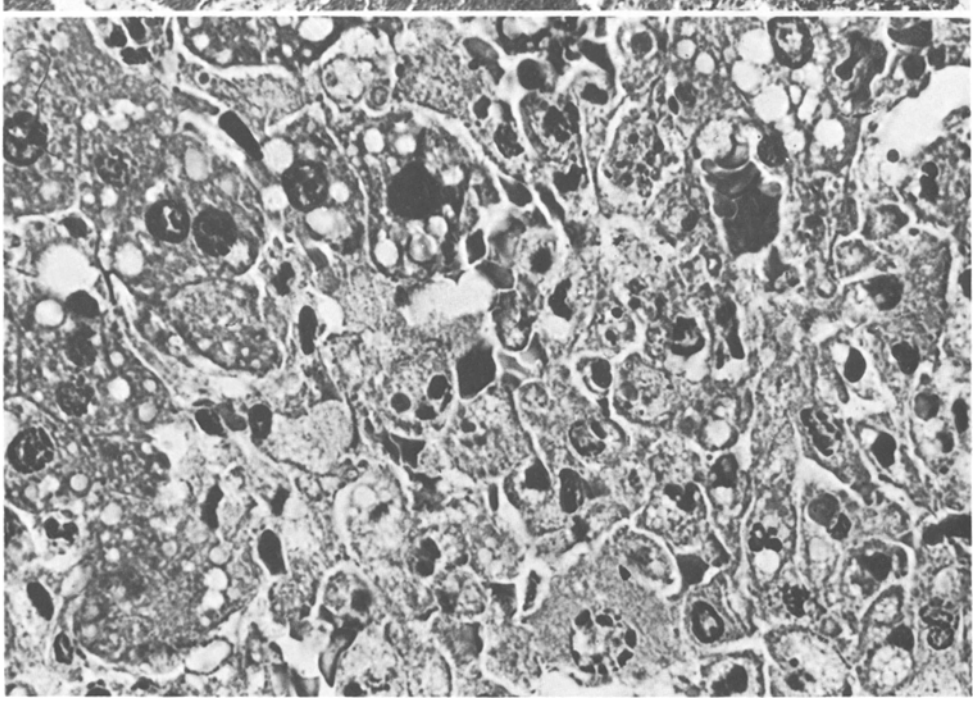

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Fig. 11. Haemangioendotheliosarcoma of the spleen (rat) following i.v. injection of thorotrast. (Photogram; HE)

Fig. 12. Kupffer cell hyperplasia (rat) following i.v. injection of thorotrast. (Photogram; HE)

Table 2. Animal experiments by Benstedt, 1963 and 1967 (explanation see text)

Tumour	ThO_2	ZnO_2	Dextrin	Contr.
1963				
Hepatoma	5	5	–	5
Adenoma of the lung	7	4	–	10
Leukaemia	4	3	3	3
1967				
Hepatoma	2	–	2	1
Hepatocell. carcinoma	1	–	1	–
Hemangioendothelioma of the liver	2	–	–	–
Angioma of the spleen	5	–	–	–
Malignant lymphoma	3	2	1	–
Adenoma of the lung	6	–	3	–

Table 3. Fabers experiment (explanation see text)

Number of animals	Activity	Results: incidence of tumours
17	1	9/17
20	7	15/20
17	49	1/7 liver cirrhosis liver dystrophy
23	Testagar	12/23
19	7 ml	16/19
22	4.5 ml	18/22
8	1.5 ml	1/8

seen more frequently amongst the dextrin animals (column 3) than amongst the controls or zirkonotrast animals. The results of Benstedt's first experiment in 1963 were completely different, in that the tumour frequency within the groups hardly varied. It seemed also remarkable that the animals of the thorotrast group showed the best general development and the biggest increase in weight of all the experimental animals, whereas the development of the zirkontrast animals was the poorest of all.

In 1973, Faber tried with a different principle in rabbit experiments to come to a satisfactory solution. The animals were given thorium-230 enriched thorotrast intravenously, and via an increase of the specific activity, Faber tried to determine the rôle of radiation effect played in tumour development.

Faber's most important experimental results are summarized in Table 3. There is evidence that by using thorotrast, tumours can be reproduced also in rabbits. However, the spontaneous tumour rate in animals treated with testagar – as shown in line 4 – is very high with 12 out of 23 animals being

affected. Faber, by increasing the normal specific activity 7-fold, was able to produce far more tumours than was the case when 'activity 1' was used (shown in the data of line 2). Unfortunately, the experimental animals in the 3rd group of experiments which employed an activity increased 49 times, died within a year from complications such as liver cirrhosis and liver dystrophy. Only 1 out of 17 animals developed a malignant haemangioendothelioma.

In the second part of this experiment, Faber injected various amounts of thorotrast with a fixed rate of activity into rabbits and was also able in this way to reproduce tumours. The decrease of tumour frequency of those animals that were given 1.5 ml thorotrast compared with the group that received 4.5 ml thorotrast was remarkable.

Faber, like others, found it impossible to establish the foreign body effect of thorotrast in these experiments.

In the experiment of the working group "Foreign body effect of thorotrast" that was mentioned earlier on, we took up Faber's principle.

It is too early to formulate a statistically relevant statement, since not all the animals have died yet and therefore, obviously, they have not yet been histologically diagnosed.

At this point, I would like to give an account of the variety and frequency of tumours that we found in 300 rats. All animals had died and were histologically diagnosed:

Fibrosarcoma with and without metastases –	14 times
Haemangioendothelioma with and without metastases –	16 times
Gall duct carcinoma –	10 times
Fibroadenoma of the mammarial gland –	6 times
Schwannoma with metastases –	twice
Teratoma –	twice
Malignant lymphoma –	12 times
Cystadenocarcinoma of the ovary with metastases –	once
Polymorphous sarcoma –	3 times
Adenoacanthoma of the uterus –	once
Stromasarcoma of the uterus –	twice
Liposarcoma of the uterus –	once
Hepatoma –	3 times
Hepatocellular carcinoma –	twice
Kupffer cell sarcoma –	4 times

The conclusions drawn from these animal experiments and their results can be summarised as follows:

1. Thorotrast or thoriumdioxyd is stored in the same fashion as in human patients in the RES where it displays its main effects.

The storage is identical with that of trypanblue and other vital dyes (Ribbert, 1904; Goldman, 1909, 1910; Aschoff, 1924; Papacharalampous, 1960; Grampa and Ferrario, 1967).

2. Thorotrast also causes tumours in animals. This is not an unimportant observation, because some researchers have concentrated almost exclusively on the fibrogenetic effect of the contrast medium, and they have emphasised that their experimental animals had not developed tumours even two years after the injection (Rüttner, 1959).

Table 4. Results of tumour generation

Author(s) Year	Animal	Number of animals	Application	Injected volume	Frequency of injection	Time of observation	Results
Andervont and Shimkin, 1940	Mouse	36	i.v.	0.2–0.8 ml	1 ×	3–6 months	Control animals and test animals with the same number of lung tumours
	Mouse	9	i.p.	0.2 ml	1 ×	9–14 months	Control animals and test animals with the same number of lung tumours
	Mouse	19	s.c.	0.2 ml	1 ×	15–18 months	2 test animals with spindle cell sarcomas, 1 test animal with a haemangioma
Benstedt, 1963	Mouse	I.16 II.18 III.18	i.v.	0.1 ml	1 ×	18 months	I. *Thorotrast:* Adenomas of the lungs: 7 Leucemia: 4 Hepatomas: 5 II. *Zirkonotrast:* Adenomas of the lungs: 4 Leucemia: 3 Hepatomas: 5 III. *Control-group:* Adenomas of the lungs: 10 Leucemia: 3 Hepatomas: 5 I. *Thorotrast:* Hemangioendothelioma of liver and spleen: 1 Fibrosarcoma at the site of injection: 1

Benstedt, 1967	Mouse	I.30 II.15 III.24 IV.20	i.v.	0.1 ml	1 ×		I. *Thorotrast:* Autopsy of 16 animals Adenomas of the lungs: 6 Lymphomas: 3 Hepatomas: 2 Hepatocell. Carcinoma: 1 Angiomas of the spleen: 5 Hemangioendtheliomas: 2 II. *Zirkonotrast:* Autopsy of 11 animals Lymphomas: 2 III. *Dextran:* Autopsy of 19 animals Adenomas of the lungs: 3 Breast tumors: 1 Hepatomas: 2 Hepatocellular carcinomas: 1 Lymphomas: 1 IV. *Control group:* Autopsy of 6 animals Hepatomas: 1	
Brunner and Rüttner, 1957	Rat	20	i.p.	0.5 ml	1 ×	2 years	The fibrogenetic effect of thorotrast on the peritoneum is the same as that of quartz. No tumours after 2 years of latency period	
Bogliolo, 1937	Mouse	30					23 test animals developed fibrosarcomas with metastases (cited by Grampa)	
Faber, 1973	Rabbit	97	i.v.	1.0 ml	1 ×	147 weeks	*Group alpha-act. 1 × :* Frequency of tumours: 9/17	I
		20		alpha-activity × 7	1 ×	103 weeks	*Group alpha-act. 7 × :* Frequency of tumours: 15/20	II
		17		alpha-activity × 49	1 ×	94 weeks	*Group alpha-act. 49 × :* All animals died within 1 year after injection. Cirrhosis and fibrosis of the liver	III
		23		Testagar	1 ×	136 weeks		IV

Table 4 (continued)

Author(s) Year	Animal	Number of animals	Application	Injected volume	Frequency of injection	Time of observation	Results
		19	i.v.	7.0 ml	1 ×	187 weeks	
		22		4.5 ml	1 ×	239 weeks	
		8		1.5 ml	1 ×	315 weeks	
		22		0.3 ml	1 ×	249 weeks	Shortening of surviving time V
Foulds, 1939	guinea pig		s.c.			3 years	Carcinomas Sarcomas
Gilman and Ruckerbauer, 1962	Rat Mouse	61	i.m.	10 mg 30 mg	1 ×	457 days	4 test animals with malignant lymphomas. No tumours in the area of the injection site
Grampa and Severini, 1963/64	Rat	50	i.v.	0.2 ml	1 ×	killing at various intervals after injection	3 test animals with liver cell hyperplasia 1 test animal with a liver cell adenoma (benign hepatoma)
	Rat	50	i.v.	0.2 ml	1 ×	killing at various intervals after injection	additional injection of estrogen: 9 test animals with liver cell adenomas (benign hepatomas) 2 test animals with hepatocellular carcinomas
Guimaraes et al., 1955	Mouse	83	i.v.	0.1 ml 0.3 ml 0.5 ml	1 ×	survival experiment	Control animals without any tumours. Test animals (with and without additional x-ray radiation): No liver cirrhosis. Proliferation of the Kupffer cells. 5 animals with benign hepatomas (same morphology as in cases with butter yellow). 1 animal with malignant histiocytoma. 7 animals with lung tumours.

							1 animal with malignant haemangioendothelioma of the spleen.
Guimaraes and Lamerton, 1956	Mouse	50	i.v.	0.1 ml	1 ×	6–12 months	In all control animals and in all test animals no cirrhosis of the liver. 2 control animals with hepatocellular adenoma (benign hepatoma). 4 control animals with adenomas of the lung. Numerous control animals with reticuloendothelial proliferation in the liver. 5 test animals with hepatocellular adenomas (benign hepatomas). 2 test animals with malignant histiocytomas. 8 test animals with adenomas of the lung.
Johansen, 1955	Rabbit		i.v.		1 ×		No tumours in controls. Fibrosarcomas, rhabdomyosarcomas, alveolarcell carcinomas; 15 reticuloendotheliosarcomas in spleen, liver and lungs.
1960	Rabbit		i.v.	45 mg–10 g/ kg weight	1 ×		Reticuloendotheliosarcoma with metastases in spleen, liver and lungs.
1967	Rabbit		i.v.	0.4–12 ml	1 ×	up to 6 years	67 test animals out of 131 with dissiminated reticuloendotheliosarcomas. 5 animals with fibrosarcomas. One animal each with a thymoma, a papilloma of the urinary bladder, a syringadenoma. 4 control animals with adenocarcinoma of the uterus. 1 control animal with breast cancer.
Mervennée and Ten Thije, 1939	Rabbit		i.v.			31–36 months	Tumours of the spleen Polymorphous sarcoma of the liver

Table 4 (continued)

Author(s) Year	Animal	Number of animals	Application	Injected volume	Frequency of injection	Time of observation	Results
Mori et al., 1966	Syrian hamster						Spindle-cell-sarcoma
Mori et al., 1973	Syrian hamster	20	Submucous	I.0.1 ml II.0.2 ml III.0.4 ml IV.0.6 ml	1 ×	More than 250 days	I. Spindle-cell-sarcoma: 3 animals II. Spindle-cell-sarcoma: 2 animals III. Spindle-cell-sarcoma: 2 animals IV. Spindle-cell-sarcoma: 0 animals
		20	Submucous	Controls	1 ×		Control-group: no tumours
		30	i.v.	I.1.0 ml II.1.5 ml III.2.5 ml	1 × 2 × 3 ×		I. Gall-duct-hyperplasia: 5 II. Gall-duct-hyperplasia: 3 III. Gall-duct-hyperplasia: 1 I. Cholangiocellular-carcinoma: 1 II. Cholangiocellular-carcinoma: 1 III. Cholangiocellular-carcinoma: 1
Miyamoto 1939	Rat		i.p. s.c.	0.5 ml	5 ×	9 months	Sarcomas
Okamoto, 1974	Syrian hamster	10 10 10	i.v.	I. 1.0 ml II. 1.5 ml III. 2.5 ml	1 × 1 × 1 ×		I. 5 adenomatous hyperplasia of the bile ductules II. 3 adenomatous hyperplasia of the bile ductules III. 1 adenomatous hyperplasia of the bile ductules I. 1 cholangiocellular carcinoma II. 1 cholangiocellular carcinoma III. 1 cholangiocellular carcinoma
Onufrio, 1938	Rabbit		i.v.			2 years	Reticulumcell-Sarcoma
Roussy et al., 1934	Rat	50	i.p. s.c.	0.1 ml	5 ×		i.p.: fibrosarcomas s.c.: fibrosarcomas and polymorphous sarcomas.
Prussia, 1936	Rat	14	s.c.	0.1–0.3 ml	15 ×		Sarcomas

Roussy et al., 1936	Chicken	5 5	s.c. i.v.	0.5 ml 0.5 ml	5× 15×	2 years	Some of the chicken had died two years after the start of the experiment, some were still alive; none of them had developed any tumours.
Roussy et al., 1936	Mouse	6 10 14	i.v. s.c. i.p.	0.25 ml 0.5 ml 0.25 ml	1× 1× 1×	1 year	All test animals remained free of tumours within the first year. Most of the test animals died within the first year. No tumour development in the area of the injection site.
Roussy et al., 1936	Rat	10	i.p.		5×	14 months	i.p.: 2 test animals out of 3 had developed a sarcomatosis of the peritoneum 1 year after starting the experiment.
		10	s.c.		5×		s.c.: 3 test animals out of 4 had developed a sarcoma.
		20	s.c.	0.5 ml			s.c.: 5 animals had developed sarcomas of the skin.
		20	i.p.				i.p.: 3 animals had developed sarcomas of the peritoneum.
		20	i.v.	0.25 ml			i.v.: All animals died within one year without having developed any tumours. All other test animals died before having developed any tumour.
Selbie, 1936, 1938	Rat	60	s.c.	0.3 ml	2×	$14^1/_2$ months	Fibrosarcomas
	Rat	60	s.c.	0.3 ml	2×	52 weeks 62 weeks 68 weeks 73 weeks	1 fibrosarcoma 13 fibrosarcomas 7 fibrosarcomas 4 little tumours
	Mouse	60	s.c.	0.1 ml	2×	39–75 weeks 71 weeks 74 weeks 94 weeks	6 fibrosarcomas 1 osteosarcoma 1 histiocytoma 1 capillary haemangioma

Table 4 (continued)

Author(s) Year	Animal	Number of animals	Application	Injected volume	Frequency of injection	Time of observation	Results
Shibata, 1966	Mice of various strains	8	i.v.	0.0 ml	1 ×		1 adenoma of the lung
		7	i.v.	0.1 ml	1 ×		2 liver cell adenomas 1 adenoma of the lung
		6	i.v.	0.3 ml	1 ×		1 liver cell adenoma
		4	i.v.	0.5 ml	1 ×		2 adenomas of the lung
		6	i.v.	0.0 ml	1 ×		1 spindle cell sarcoma
		8	i.v.	0.3 ml	1 ×		3 liver cell adenomas 1 Kupffer cell tumour
Swarm et al., 1962	Rabbit	3	i.v.	3.0 ml	1 × 3 rabbits 2. inject. after 18 days	37 months	2 animals with 2 injections had developed hemangioendotheliomas of liver and spleen
		9			1 ×	54 months	1 hemangioendothelioma 8 animals without tumors No animal with cirrhosis or fibrosis of the liver
Wenz, 1964	Rat	300	i.p.	25 mg 50 mg 250 mg 500 mg 1000 mg	1 ×	1.5–2.5 years	*Tumours of thorotrast-animals: 84* Tumours at the site of inject.: 57 Tumours of the liver: 10 Tumours of soft tissue: 9 Tumours of the sceleton: 4 Tumours of the ovary: 2 Tumours of the mesenerium: 2 *Tumours of control-animals: 21* Tumours of soft tissue: 10 Tumours of the mesenterium: 7 Tumours of the uterus: 1 Tumours of the thymus: 1 Tumours of the pancreas: 1 Tumours of the adrenal gland: 1

							Sort of tumours: Hemangioendotheliosarcoma of the liver Spindlecell-Sarcoma of the liver Hepatocellular carcinoma with metastases Spindlecell-Sarcoma at the site of inject. Osteoid-Sarcoma at the site of inject. Osteoid-Sarcoma of the humerus Fibro-osteoid-Sarcoma
Working Group Foreign Body Effect of Thorotrast	Rat	2000	i.v.	Different Volumes	1 ×	Survival	Different tumors in many organs The experiment is ongoing
Zeitlhofer and Speiser, 1954	Rabbit	1	i.v.	10 ml	1 ×	4 years	Hemangioendothelioma of liver, spleen and lungs

3. All the tumours which occur after the injection of thorotrast are known to occur spontaneously in animals too (Bullock and Rohdenburg, 1917; Ratcliffe, 1940; Crain, 1958).

4. Only in a few cases did animals show alterations of the liver such as cirrhosis. This is a clear difference from the results found in men. In cases of human thorotrastosis, the point is always stressed that an indefinable number of hepatocellular and cholangiocellular carcinomas have developed from a precancerosis, that is to say from "liver cirrhosis".

5. As in men, tumours of the spleen or malignant lymphomas are seldom found.

6. According to the investigations by Wenz (1964), the formula 'c.t=constant' does not apply to thorotrast. That is to say, the product of concentration and latency period is not constant. This contrasts with the results of investigations into the generation of hepatocellular carcinomas using butter yellow (Druckrey and Küpfmüller, 1949).

In the experiment by Wenz (1964) the number of tumours increases with increasing concentration, that is to say with increasing alpha-activity.

7. Other investigators, for instance Faber in 1973, did not see an increase in the number of tumours, but noted a shorter latency period.

8. None of the experiments so far carried out have brought a satisfactory and statistically safe answer to the question of how extensive the effect of the factor "foreign body influence" is during the tumour development. Equally, the extent of the effect of "radiation" is not known.

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