STUDY OF RETICULO ENDOTHELIUM OF THE RAT LIVER FOLLOWING THE INJECTION OF THORIUM DIOXIDE AND FEEDING OF PARADIMETHYLAMINOAZOBENZENE (BUTTER YELLOW)

by

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The phagocytosis of thorium dioxide by the reticulo endothelial system has made possible extensive experimental and clinical diagnostic hepatosplenographic studies following the intravenous injection of thorotrast. The actual toxicity of the thorotrast has been a point of considerable dispute.

P-dimethylaminoazobenzene (butter yellow) administered in the diet of albino rats is a known carcinogenic agent.

With these thoughts in mind experimental work was done to study the following.

1. The phagocytic activity of the reticulo endothelial cells of the liver, Kupffer cells, as demonstrated by their ability to engulf thorium dioxide and the initial and subsequent distribution of phagocytized particulate matter.

2. The effect of varying dosages of thorotrast on the liver.

3. The effect on the liver of an addition of .06 percent p-dimethylaminoazobenzene (butter yellow) to the diet for a period of sixteen weeks or less.

4. The combined effect of thorotrast and butter yellow on the liver.

The above studies were carried out on white rats thorotrast being injected into the tail vein and butter yellow being added to the diet.

Larger series of rats and longer periods of study would have been desirable and any conclusions drawn must be considered in this light.

Thorotrast is a 25 percent colloidal suspension of thorium dioxide in a 16-18 percent aqueous dextrin solution. Thorium dioxide was used in this experiment because of several desirable properties of this substance; phagocytosis by the cells of the reticulo endothelial system, relatively inert chemically, radio opaque, easily identified under the microscope, only slightly radio active and for all practical purposes not eliminated from the body.

Thorotrast was used with good success beginning in 1929 for diagnostic purposes but has been used only sparingly in recent years because of the question of harmful effects due to its radio activity. The Council of Pharmacy and Chemistry (1) in 1932 voted thorotrast not be accepted for human use because of its radio activity which they showed to be as follows:

Alpha ray, 25 cc. of thorotrast equivalent to 1 microgram of radium.

Beta ray, too feeble to be of physiological significance.

Gamma ray, 25 cc. of thorotrast equivalent to .4 microgram of radium.

Dickson (2) in 1932 gives some of the clinical uses of thorotrast which include retrograde pyelogram, diagnosis of cirrhosis, cysts, abscesses and metastasis of the liver,

diagnosis of cysts, tumors and enlargement of spleen, visualization of placental outline and arteriography. It is interesting to note that Dickson's article published three months prior to the adverse report of the Council of Pharmacy and Chemistry states that he believes thorium dioxide to be an absolutely harmless contrast medium and invaluable in diagnosis of certain lesions in the living subject. Other authors, Macdonald, Dudley and Irwin (17-4) also claimed no ill effects in use of thorotrast.

Yater and Coe (29) in 1943 report a follow up study on 286 persons who had received thorotrast over a ten year period. They state that there is no immediate or remote ill effects noted and no evidence of depression of hepatic, splenic or hematopoietic function from the use of thorotrast. They conclude that the use of thorotrast in hepatosplenography is of definite value in helping diagnose cirrhosis of the liver, to determine presence of metastasis in liver and in diagnosis of abscess of the liver. However, Jacobson, Rosenbaum, Gottlieb, Orr, Popoff, Rosedale, Stephenson, Tripoli, Haam (14-9-22-27) and others all give evidence either in experimental animals or in clinical study that thorium dioxide is actually harmful.

CONTROL SERIES

Control series for histological purposes consisted of two normal rats on stock diet who received no thorotrast or

butter yellow and four rats on a synthetic diet receiving butter yellow but no thorotrast.

NORMAL RATS ON STOCK DIET

The histological sections of the rats liver reveal it to be in all essentials similar to that of the human liver. The structural unit being the hepatic lobule with its central vein, cords of liver cells radiating out from it to the periphery of the lobule and between the cords of cells are seen the intralobular capillaries (sinusoids). The bile capillaries occur between the opposed surfaces of the hepatic cells. The portal triad is seen as in the human liver with its portal vein, artery and bile duct or ducts. The only real difference observed other than size was that in one of the two normal livers observed there was a definite lobulation of the surface. Xray of the normal rat does not show outline of the liver (Fig. 10B).

Hans Elias (5) recently (1949) in extensive studies of the liver feels that the long accepted hepatic cord structure theory of the liver is no longer tenable. He feels that the mammalian liver is fashioned of cribiform sheets or plates one cell thick which anastomose with one another and between them are the sinusoidal spaces. The few sections studied in this series would neither substantiate or disprove either the cord or sheet theory but would conform with either one. However this theory which is not new with Elias, but new to

most readers, is well substantiated by his studies and excellent illustrations are to be found in his article.

CONTROL RATS ON BUTTER YELLOW DIET BUT NO THOROTRAST*

Four rats were studied in this group being on control diet to which .06 percent p-dimethylaminoazobenzene had been added as follows:

Rat 1 21 days on butter yellow diet then sacrificed.

Rat 2 77 days on butter yellow diet then sacrificed.

Rat 3 16 weeks on butter yellow diet and 8 weeks on control diet then sacrificed.

Rat 4 Same as rat 3.

At the end of twenty one days there is cloudy swelling of the liver cells; otherwise the liver appears normal. By the end of ten weeks the liver structure is fairly well maintained but there is considerable fatty degeneration and necrosis seen which is more marked on the periphery of the liver. In the lobules in which changes are occurring the changes are more marked at the periphery. Beginning cirrhosis is seen with areas of regeneration. A moderate increase of fibrous connective tissue is present. In the twenty four week specimens moderate nodularity is grossly apparent. Microscopically a variable picture is observed.

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* Acknowledgment is made to Charles C. Clayton for injection and feeding of the animals.

There are some areas of fatty degeneration and necrosis. Marked cirrhosis is apparent with regeneration of fairly normal appearing cords of liver cells, but the cords are arranged in no definite pattern. There is marked increase of perilobular fibrous connective tissue, and only a few central veins and portal triads are seen with the normal relation to liver cells.

Sections from a tumor area show no structural pattern. There are large lakes of homogenous eosinophilic staining material, fatty degeneration, necrosis, no normal liver cells and a haphazard arrangement of all cells are observed with a beginning apparently benign bile duct proliferation.

In summary this series suggests that with a continuing diet containing butter yellow cirrhosis occurs; gradually becoming more severe.

Steinberg and Martin (23) found an absorption of connective tissue and partial or complete return to structural integrity following return to normal diet after diet with butter yellow. In rats studied in this experiment there is no evidence of return to normal and from the marked changes occurring it does not seem likely that there would be, but the time element following cessation of butter yellow was not long enough to make a favorable comparison. Also in this series there is a suggestion of neoplastic change resulting from continued butter yellow diet evidenced by bile duct proliferation which, however, at this point still appears fairly benign.

RATS ON CONTROL DIET AND 1CC OF THOROTRAST PER KILOGRAM

This series of 13 rats on control diet received 1cc per kilogram of thorotrast by tail vein injection and were killed by illuminating gas subsequently from two hours to thirty days following injection. Immediately after being sacrificed they were xrayed.

Histologic studies revealed that the particulate thorotrast was phagocytized by the Kupffer cells of the liver very rapidly, and it was assumed that other parts of the reticulo endothelial system similarly phagocytized the thorotrast.

By the end of two hours at which time the first animal was sacrificed histological sections reveal particulate matter in most of the Kupffer cells with a peppering of thorotrast throughout the liver but with a tendency to concentration in the area of the portal triad. Six hour specimen reveals a similar picture with the Kupffer cells still retaining their characteristic flattened shape. By twenty four hours the phagocytic cells are becoming more engorged and rounding out with a tendency for thorium dioxide to occupy one pole and the nucleus the other. Many of the cells have lost their sinusoidal attachment and appear free. By the end of six days there is a definite tendency for more

marked concentration in midzone area. Increased clumping and many multinucleated giant cells are seen with increase of time up to thirty days when there appears to be complete plugging of some sinusoids. In many instances the thorium dioxide appears to be free in the sinusoids suggesting a rupture of the Kupffer cells.

Under oil immersion in the thirty day specimen there is still particulate matter seen in most of the Kupffer cells and for the first time particulate matter is seen in a very few parenchymal cells. It is interesting to note that with the exception of one slide in a three day specimen no particulate matter is seen in portal or central veins. However, as many of the vessels appeared completely empty the actual picture may be distorted.

Several animals in this series had received a tail vein injection of india ink two weeks prior to the injection of thorotrast. These animals were sacrificed at the following intervals following injection of thorotrast 2, 8, 12, 16 and 20 hours. The thorium dioxide was phagocytized very readily by the Kupffer cells already containing large amounts of india ink showing clearly that phogocytosis of one material does not prevent another material from being taken up by the same cells. (Figure 3). The most striking features of this series are

First the rapidity of phagocytosis by the Kupffer cells. Second a dynamic and continually changing picture with first

a diffuse peppering of thorium dioxide changing by fifteen days to a definite picture of midzone clumping which is more pronounced at the end of thirty days. There is apparently a pattern of migration or distribution of the material developing which is not definite at this time.

Roentgenographic studies showed a diffuse increased density well outlining the liver and spleen at the end of 6 hours (Fig 1). More definite at the end of 24 hours. (Fig. 2) By the end of thirty days there is a definite change in appearance of the liver which is evidenced by a tendency to a finely granular appearing increased density.

Yater (28) in his clinical studies points out that the phagocytic cells in the liver and spleen engulf sufficient thorium dioxide in fifteen minutes to cast a shadow on xray. This results following injection of .5cc of thorotrast per pound up to 75cc in one total dosage and two hours later all definition necessary will be present for diagnosis.

Maxfield and Mortensen (18) in an experiment on rabbits studied the disappearance of thorium dioxide from the circulating blood and found that following injections of 1cc per kilogram 85 percent was removed in the first four hours and by the end of five and one half hours more than 99 percent had been removed.

RATS ON CONTROL DIET WITH VARYING DOSAGES OF THOROTRAST

This group of seven rats was given dosages of thorotrast ranging from 1½ to 4cc of thorotrast per kilogram and covering a period of from one day to twenty four weeks. Thorotrast 1cc per kilogram was injected in the tail vein at the start and ½cc per kilogram every fourteen days thereafter until 4cc had been given unless the animal was sacrificed previous to that time. Thorotrast was given at these intervals to have it correspond to the intervals of injection of the rats on butter yellow diet and varying dosages of thorotrast.

This series as in the preceding series shows first a diffuse peppering with a tendency to periportal concentration then a periportal and midzone concentration and in ten week specimen some tendency to central vein concentration with a definite central vein collaring in the twenty one and twenty four week specimens. There is also a marked concentration in the area of the hepatic artery in the latter specimens with a tendency but not as pronounced collaring as in the case of the central vein.

At ten weeks there is beginning degeneration of liver parenchyma seen. In twenty one week specimen there is marked fatty degeneration and some necrosis of liver cells,

but the structural appearance of the liver is still intact. In the twenty four week specimen the structural outline of the lobules are lost with the only means of orientation being the portal triads and central veins. Large areas of fatty degeneration and hepatic cell necrosis are present and practically no normal liver cells are observed. No attempt at regeneration is evident in the sections studied. There are large areas free of thorium dioxide the concentration being perivascular. There appears to be a paucity of thorium dioxide considering the amount injected suggesting a loss of the material from the liver. Tripoli and Haam (27) show a loss of much thorotrast from the liver at the end of six months. Irwin (13) shows a migration of thorotrast to the central vein and a subsequent loss from the liver.

Xray studies of the one day specimen reveal the liver to be well outlined. At eighteen weeks the liver is well outlined with a marked granularity to its appearance. Axillary and inguinal lymph nodes stand out prominently.

From the above findings it would seem that thorotrast in amounts given is a definite destructive liver agent either on the basis of foreign body reaction, radio activity or both. Actual cirrhosis as described by other experimenters, Harris and Friedrichs (12) is not confirmed.

RATS ON BUTTER YELLOW AND VARYING DOSAGES OF THOROTRAST

This series includes nine rats receiving butter yellow and thorotrast. Butter yellow .06 percent was added to a diet consisting of

(1) extracted casein 12 percent

(2) salts 4 percent

(3) corn oil 5 percent

(4) glucose monohydrate to 100 percent.

Vitamins were added at the following level in mg. per kilogram of diet. Thiamine 3, pyridoxine 2.5, calcium pantothenate 7.5, riboflavin 1, and choline 30 and two drops of halibut liver oil were given to each animal every four weeks. Butter yellow was added for sixteen weeks unless the animal was sacrificed prior to that time and then the synthetic diet was fed for an additional eight weeks at which time all remaining animals were sacrificed. Thorotrast 1cc per kilogram was given initially and an additional ½cc at two week intervals until a total of 4cc had been injected. This was done with the thought of blocking or partially blocking the reticulo endothelial system. Gottlieb (7-8-9) in his studies showed that thorotrast 6cc per kilogram completely blocked the function of bilirubin formation in the reticulo endothelial system for periods up to ten days.

At the end of fourteen days concentration of thorium dioxide was in midzone and portal area. Some cloudy swell-

ing of liver cells is present. In the seventy day specimen there is marked fatty degeneration, necrosis and increased fibrous connective tissue. Moderate cirrhosis with regeneration is seen and there is beginning bile duct proliferation which appears to be fairly benign. The thorium dioxide has migrated to show a marked perilobular concentration as well as a vascular collaring with only minute amounts in areas of necrosis and regeneration. Kelty, Baggenstoss and Butt (15) suggest that with necrosis of the lobule there is a collapse of the sinusoids and reticular framework and with regeneration the collapsed sinusoids and reticular framework are pushed out to the periphery of the lobule. This would well explain the perilobular concentration of the thorotrast and the paucity of the thorotrast in the lobule as well as the increased perilobular fibrous connective tissue.

With an increase of time to twenty three and twenty four weeks one of the livers shows a large tumor. Sections from the tumor area reveal entirely new growth in the form of an adenocarcinoma with definite gland like structure arising from the bile ducts. (Fig. 4) Many mitotic figures are seen and this is a malignant growth apparently a cholangioma. Thorium dioxide particles in the tumor sections are seen only under oil immersion and these are few. Other sections of this liver and other twenty four week specimens show moderate to marked cirrhosis. Thorotrast in the cirrhotic areas again is perivascular and perilobular.

Roentgen studies of this series show liver well outlined with minimal granularity at sixteen days. In two ten week specimens one shows a very coarse granularity (Fig. 19) indicating liver damage while the other shows a fine granularity suggesting little liver change. The liver is well outlined in both specimens. The one twenty four week specimen shows a very coarse granularity and a large well circumscribed less dense area at the site of a tumor (Fig. 21-H). Marked damage is suggested elsewhere. Other twenty-four week specimens shows marked granularity indicating damage. (Fig. 33 and 29)

Roentgen studies made at regular intervals following injection of thorotrast would make possible the study of progressive changes in the liver without the necessity of sacrificing the animal for histologic studies until the desired roentgen picture was present.

In reviewing the above series it is apparent that the combination of thorotrast and butter yellow diet in dosages given are definitely damaging to rats' livers causing cirrhosis in all rats carried to twenty four weeks and in one rat apparently resulting in a cholangioma. Whether either agent enhances or inhibits the other or whether there is a synergistic effect of the two agents cannot be determined from this limited series, but the author feels that the probability of a combined effect is more reasonable because the histologic changes are more marked here than in the

controls on either thorotrast or butter yellow diet alone.

SUMMARY

1. Thorotrast is rapidly phagocytized by the Kupffer cells of the liver as evidenced by histologic and roentgen studies.

2. A dynamic and continually changing picture of thorium dioxide distribution is seen with first a diffuse peppering later a midzone and perivascular concentration and still later a more pronounced perivascular concentration with a suggestion of loss of thorotrast from the liver at the end of twenty four weeks.

3. Thorotrast in amounts given was definitely harmful to the liver.

4. A continuing diet containing .06 percent butter yellow causes a cirrhosis gradually becoming more severe with a suggestion of neoplastic change in one specimen.

5. A combination of thorium dioxide and butter yellow are definitely damaging to the rat's liver resulting in cirrhosis in all animals carried for twenty four weeks and causing a cholangioma in one specimen and suggestion of neoplastic change in another.

6. Thorotrast is an excellent medium for roentgenographic diagnosis of liver damage and tumor formation in the experimental animal.

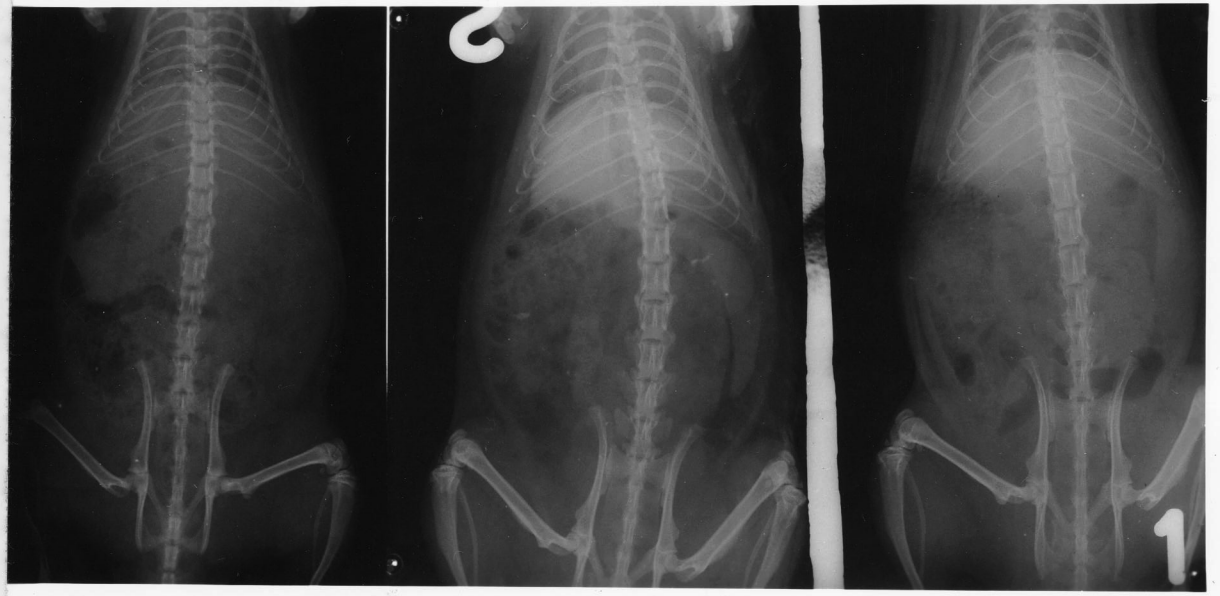

Figure 10b

Figure 2

Figure 1

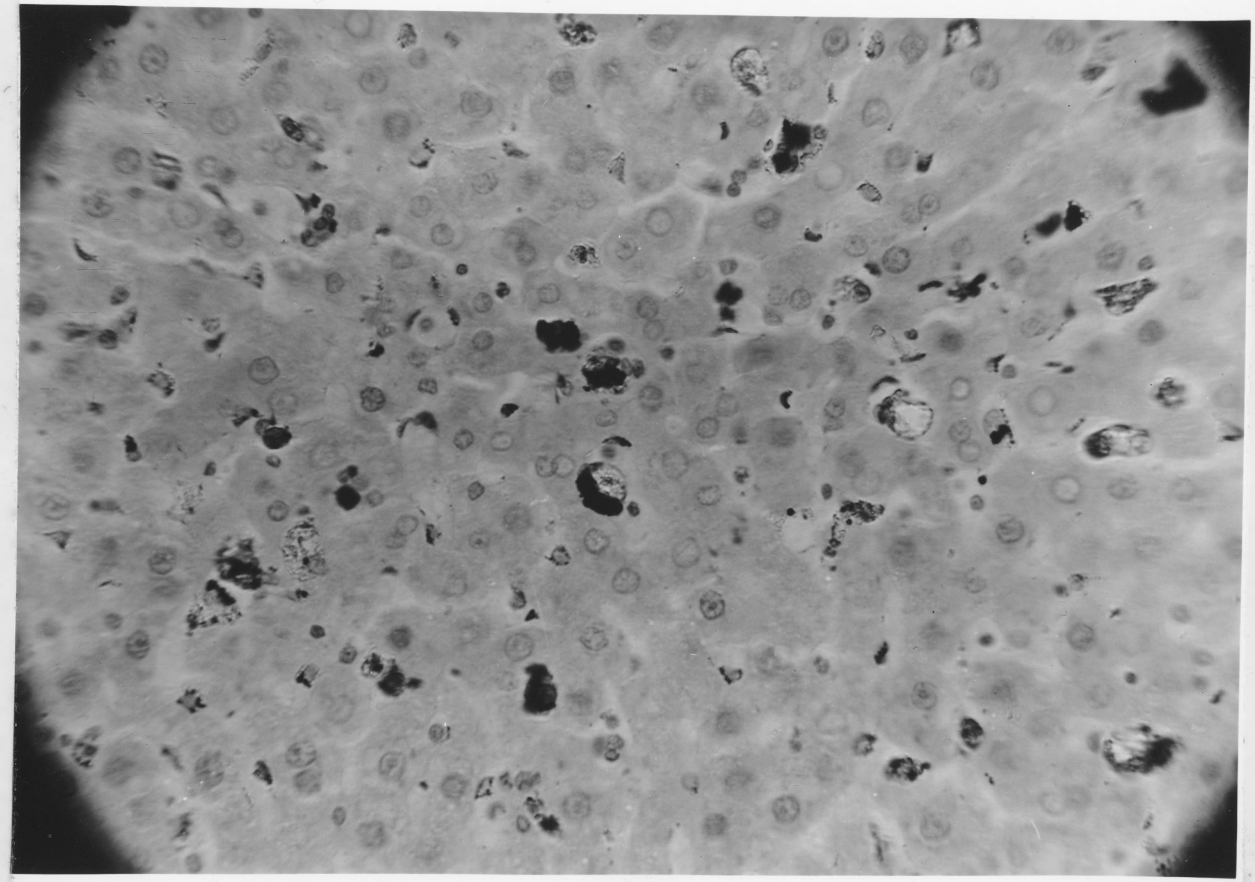

Figure 3

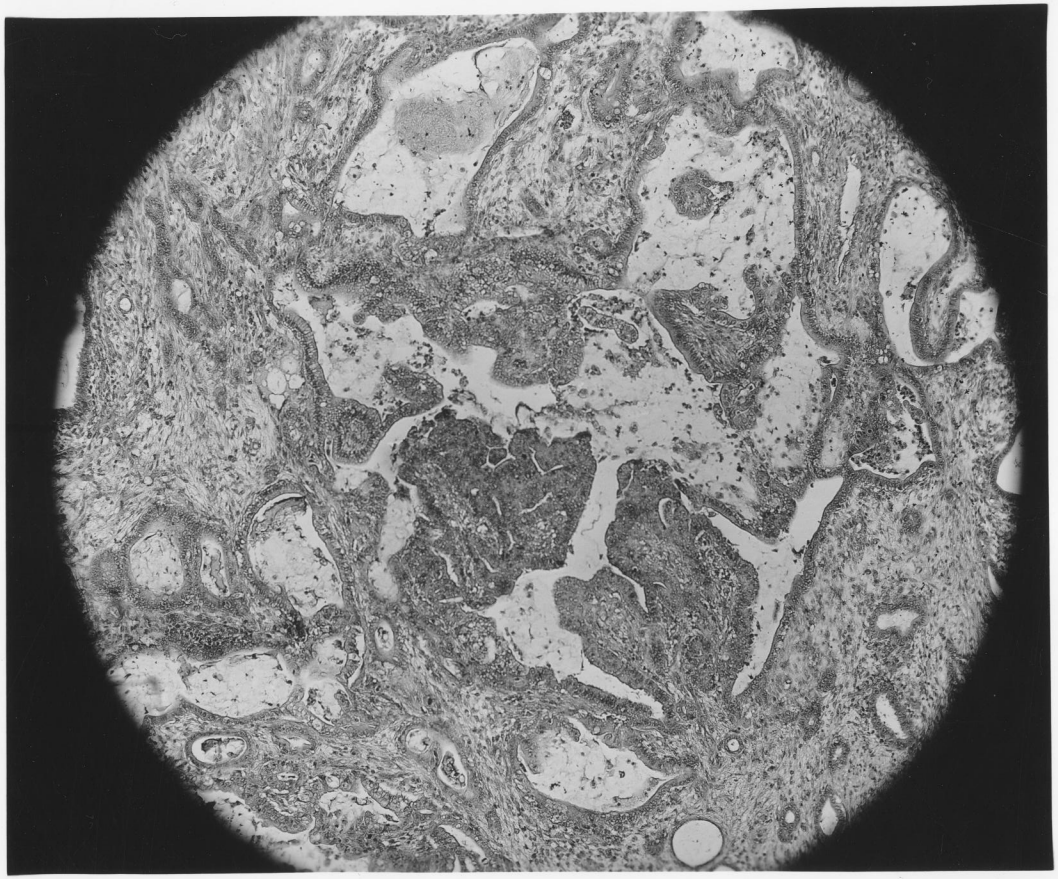

Figure 4

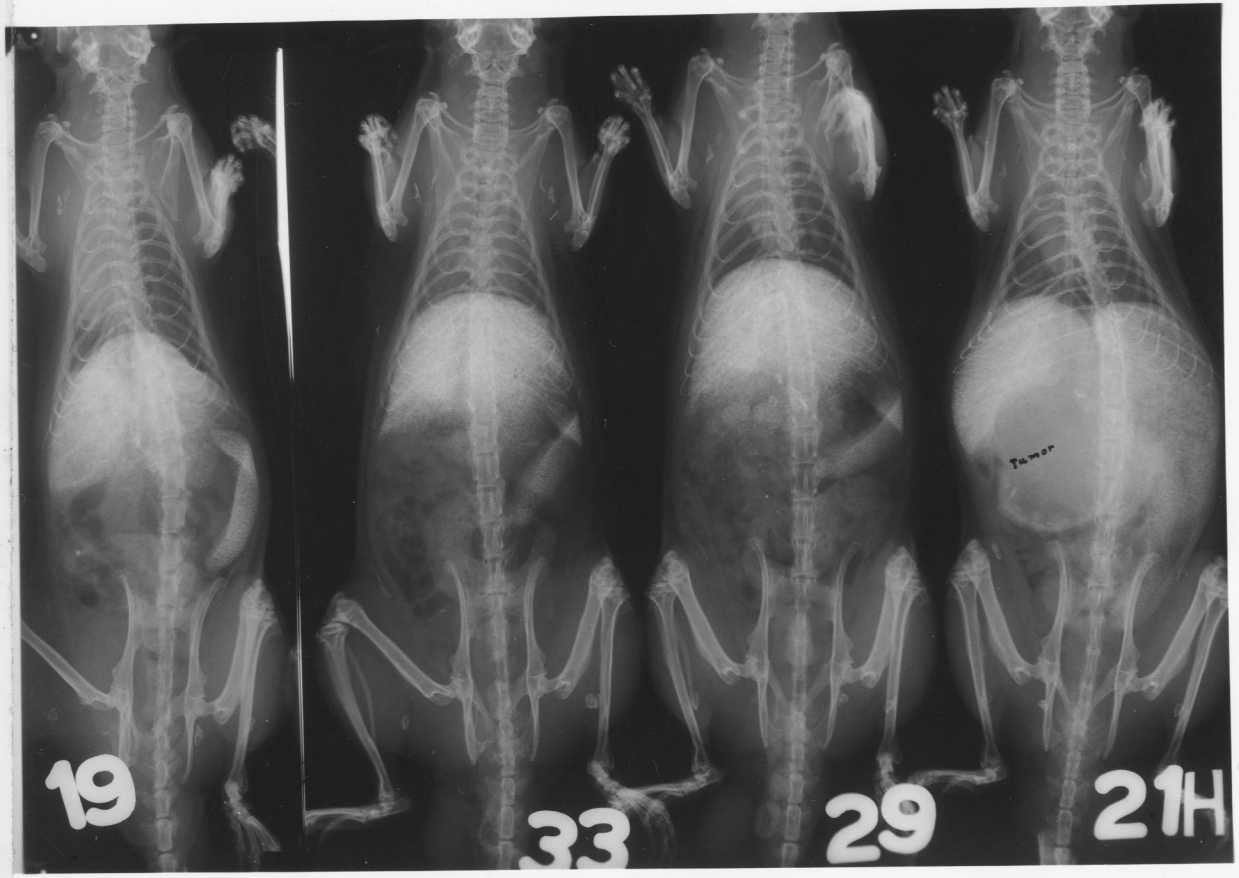

Figure 19 Figure 33 Figure 29 Figure 21H

BIBLIOGRAPHY

1. Council of Pharmacy and Chemistry, Preliminary report on thorotrast. J.A.M.A. 99: 2183, 1932.

2. Dickson, Thorotrast (A new contrast medium for radiological diagnosis. Cand. Med. Ass. Jour. 27: 125-129, 1932.

3. Clayton, Effect of Dietary variation on the production of tumors by azo dyes. Thesis, Univ. of Wis. 1949.

4. Dudley and Irwin, Experimental intravenous administration of colloidal thorium dioxide. Cand. Med. Ass. Jour. 27: 130-136, 1932.

5. Elias, Hans, A Re-examination of the structure of the mammalian liver. I. Parenchymal Architecture, Seventeen figures. Am J. of Anatomy 84: 311-333, 1949.

6. Gottlieb, Use of colloidal thorium in clinical medicine. Cand. Med. Ass. Jour. 27: 356-360, 1932.

7. Gottlieb, Bilirubin formation and the reticulo endothelial system. I The kupffer cells and their relation to the reticulo endothelial system. Cand. Med. Ass. Jour 30: 256-258, 1934.

8. Gottlieb, Bilirubin formation and the reticulo endothelial system. II Anatomical block of the reticulo endothelial system. Cand. Med. Ass Jour. 30: 365-368, 1934.

9. Gottlieb, Bilirubin formation and the reticulo endothelial system. III Functional block of the reticulo endothelial system. Cand. Med. Ass. Jour. 30: 512-515, 1934.

10. Guest, Depostition of thorium dioxide in the cells of the reticulo endothelial system (dog). Thesis, Univ. of Wis. 1938.

11. Harris, Histo-pathological effects of thorium dioxide on lymphatic glands of animals visualized by Menville-Ane' method. Exp. Biol. and Med. 29: 1049, 1932.

12. Harris and Friedrichs, Microscopic changes produced in tissues of animals injected with thorium dioxide for splenehepatography. Exp. Biol. and Med. 29: 1047-1049, 1932.

13. Irwin, Kupffer cell migration. Cand. Med. Ass. Jour. 27: 353-356, 1932.

14. Jacobson and Rosenbaum, Postmortem findings and radioactivity determinations five years after injection of thorotrast. Radiology 31: 601-607, 1938.

15. Kelty, Baggenstoss and Butt, The relation of the regenerated nepatic nodule to the vascular bed in cirrhosis. Proc. of Mayo Clinic 25: 17-26, 1950.

16. Lohmiller, An experimental study of the reticulo-endothelial system. Thesis, Univ. of Wis. 1942.

17. Macdonald, The use of thorium dioxide as an aid to clinical diagnosis. Cand. Med. Ass. Jour. 27: 136-137, 1932.

18. Maxfield and Mortensen, The use of radioactive properties of thorium for a quantitative study of phagocytosis. Jour. App. Physics. 12:3: 197-202, 1941.

19. Menville and Ane, Roentgen-ray study on non-permeability to thorium dioxide of placenta of albino rat. Exp. Biol. and Med. 29: 1045-1047, 1932.

20. Miller, Miner, Rusch and Baumann, Diets and Hepatic tumor formation. Cancer Research. I: 699-708, 1941.

21. Pohle and Ritchie, Histological studies of the liver spleen and bone marrow in dogs following the intravenous injection of thorium dioxide. Am. Jour. of Roentgenology. 41: 950-953, 1939.

22. Orr, Popoff, Rosedale and Stephenson. A study of the effect of thorium dioxide sol injected in rabbits. Radiology 370-381, 1938.

23. Steinberg and Martin, Absorption of scar tissue in experimental nodular cirrhosis of the liver. Archives of Pathology. 41: 1-10, 1946.

24. Stenstrom and Vigners, Elimination of radioactive elements in patients who have received thorotrast intravenously. Soc. Exp. Biol. and Med. 44: 18-23, 1940.

25. Steinberg Walliker and Martin, Recognition of liver cirrhosis induced by paradimethylaminoazobenzene (butter yellow) by x-ray and thorium dioxide. Soc. Exp. Biol. Med. 55: 165-167, 1944.

26. Taft, Radioactivity of thorium dioxide sol. J.A.M.A. 108: 1779-1781, 1937.

27. Tripoli and Heam, Effects of toxic and non toxic doses of thorium dioxide in various animals. Exp. Biol. and Med. 29: 1053-1056, 1932.

28. Yater, Rate of deposition of thorotrast in the human liver and spleen. Am. Jour. Roentgenology, 38: 447-449, 1947.

29. Yater and Coe, Ten years experience with thorotrast hepatosplenography. Ann. of Int. Med. 18: 350-365, 1943.

30. Yater, Otell, and Hussey, Hepatosplenography with stabilized thorium dioxide sol. (A follow up study of 200 patients examined over a period of five years.) Radiology 27: 391-409, 1936.

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