THE EXPERIMENTAL INTRAVENOUS ADMINISTRATION OF COLLOIDAL THORIUM DIOXIDE*

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DURING the past two years many workers have published communications on the use of colloidal thorium dioxide in roentgenology, as introduced by Radt.[16] The sequelæ of intravenous injection in experimental animals have been reported by a number of investigators. In this communication, similar experimental work is reported, done during the last few months in this department. Twenty-five healthy rabbits were used in these experiments. Varying initial doses of the thorium compound were given and the animals examined at intervals. In other animals small daily doses were given for a period of two months. The clinical course and the changing density of the liver and spleen to roentgen rays were observed, and the blood and urine examined. The tissues were examined histologically. Two chickens bearing experimental Rous sarcoma were used also to ascertain the presence or absence of thorium in the malignant tissue.

Thorium dioxide (abbr. ThO_2) is a very stable compound of thorium which has the property of absorbing roentgen rays. The particular preparation used in this work was a 25 per cent colloidal solution of ThO_2 in serum, known commercially as "Thorotrast".† This preparation mixes readily with blood in any proportion without the formation of any visible clot. When alcohol is added to this preparation the ThO_2 is quickly thrown down as a white precipitate, and hydrochloric acid, which had been adsorbed to the ThO_2 to maintain its colloidal state, is freed.

REACTION TO THE INTRAVENOUS ADMINISTRATION OF THORIUM DIOXIDE

In all animals used in this work the ThO_2 solution was introduced into the marginal ear vein, undiluted, at room temperature, and as fast as a small needle would permit. No untoward clinical symptoms were observed in any of the animals during or immediately following the intravenous injection. Kadrnka[4] advised a 1-10 dilution of the preparation in serum containing 5 to 10 per cent glucose. This was given slowly at body temperature. He[5] also mentioned occasional reactions following intravenous administration, similar to a foreign protein reaction. This reaction was probably due to the serum portion of the preparation and not to the ThO_2 *per se*.

Most of the animals lost slightly in weight for a few days following administration, but this was recovered quickly. Some showed a hæmoglobin loss as high as 10 per cent that disappeared in a few days. Kadrnka[6] reported a mild transient decrease in the erythrocytes. Lambin[9] found a pronounced anæmia following single large doses, recovery being spontaneous. The degree of anæmia was less when he gave similar amounts in divided doses.

DOSAGE

Lambin[9] reported 5 c.c. per kilo. necessary for rabbit liver visualization, though the spleen in the same animal required only 1 c.c. per kilo. for comparable contrast. Kadrnka[4] found 0.8 to 1.0 grams per kilo. necessary for average contrast in rabbits. He recommended dividing that amount into 4 to 6 doses. Muramatsu[12] found 0.7 c.c. per kilo. sufficient for spleen and liver visualization in rabbits. In this experimental work it was found that proper visualization of liver and spleen in rabbits was difficult, due to a rapid respiratory rate and a relatively large stomach. If the stomach was empty and the roentgen exposure short, the liver and spleen were seen faintly when 0.25 c.c. per kilo had been given. The density of these organs increased proportionately with the dosage and good contrast was present after a total of 0.8 to 0.9 c.c. per kilo. had been given. Little

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† "Thorotrast" is prepared by the Heyden Chemical Corporation, New York, to whom we are grateful for the supply used in this work.

change was noted in the density of these organs until a total of 1.8 c.c. per kilo. had been reached. At this point the density was marked.

DISTRIBUTION OF AND TISSUE REACTION TO THORIUM DIOXIDE

Leipert[10] found the thorium exclusively in the liver and spleen, with a minimal amount in the kidney. He also reported the absence of thorium in tumour tissue. Kadrnka[7] found the thorium in liver, spleen, bone marrow, lung and adrenal glands of rabbits. Randerath[17] reported finding thorium in the human liver, spleen, bone marrow and adrenal glands, and none in carcinoma metastases. All experimenters agree that this preparation of thorium dioxide, when given in doses up to 5 c.c. per kilo. (*i.e.*, six times the dose necessary for visualization of liver and spleen), causes no untoward tissue reaction up to periods of at least one year. Huguenin[2] reported hepatitis and liver cirrhosis in rabbits following doses of 10 c.c. per kilo. Kadrnka[4] reported one animal healthy 3 months after a dose of 14 g. per kilo.

Baumann and Schilling[1] found a fading liver and spleen shadow from the 20th day after administration. Kadrnka[4] found liver contrast diminished by half at the end of 3 months. The same author[6] assumed that some thorium was eliminated through the lungs. Naegeli and Lauche[13] found no ways of elimination. Popper and Klein[14] were of the opinion that practically no elimination occurred in 2 months. Huguenin and Nemours[3] reported pronounced opacity of liver and spleen 5 months after administration.

TECHNIQUE

The animals were killed and the tissues removed immediately to 10 per cent formo-saline for fixation. As a routine, paraffin sections stained with hæmatoxylin and eosin were made of 20 of the more important organs, for microscopic examination. Frozen sections stained for fat with Sudan 3 were made of liver and kidney. ThO_2 was easily detected microscopically, and appeared as a finely granular, highly refractile, grayish bronze material of metallic appearance, in stained or unstained sections. Huguenin, *et al.*[2] found that aniline blue selectively coloured the ThO_2, but such staining was found to be unnecessary.

GENERAL FINDINGS

ThO_2, given intravenously to rabbits in doses up to 5 c.c. per kilo., was found in large amounts in the liver, spleen, bone marrow, lymphoid tissue; in small amounts in the adrenal and ovary; and in traces in the kidney. This material was found to be engulfed only by the reticulo-endothelial cells of these organs, with the exception of the parenchymatous cells of liver and adrenal. When given subcutaneously, the ThO_2 was soon engulfed by the histiocytes. The presence of ThO_2 was found to be innocuous to the containing or surrounding cells. There was no evidence of leucocytic infiltration, fatty degeneration, granulomatous reaction, fibrous proliferation, or other untoward reaction in four months after doses as large as 5 c.c. per kilo. One animal suffering from extensive coccidioidal infestation of liver showed complete absence of ThO_2 in the cyst walls or cavities. The chickens bearing experimental Rous sarcoma failed to show any ThO_2 in the malignant tissue following large intravenous doses.

Liver.—Relatively, the liver engulfed slightly less ThO_2 than the spleen. The amount present in the liver was proportional to the amount of ThO_2 given intravenously. The ThO_2 was only found in the cytoplasm of the parenchymatous and Kupffer cells.

If 1 c.c. per kilo. was given at one dose and the liver examined in 48 hours, the ThO_2 was present in the cytoplasm of practically all the parenchymatous liver cells. It was seen as small, finely granular collections distributed fairly evenly throughout the cytoplasm. There was no typical zonal distribution, though a greater concentration was occasionally seen in the liver cells of the outer zone. The majority of the Kupffer cells contained similar collections of ThO_2, but not to the extent of displacement of the nucleus or marked bulging of the cell. These Kupffer cells were distributed in a uniform manner throughout the lobule. Hyperplastic aggregations of Kupffer cells were seen in some areas. This reaction coincided with a local increase of the ThO_2 content. It was interesting to note that many of the Kupffer cells contained no ThO_2, though frequently adjacent to Kupffer cells laden with that material. Variations of the dose or the time

interval after injection apparently did not induce engulfing of ThO_2 by these cells. At this stage, with this dosage, there was no evidence of leucocytic infiltration, cloudy swelling or any other type of damage. Frozen sections, stained with Sudan 3, showed no increase in the occasional intracellular fat granules usually seen in the livers of healthy control animals. There was no ThO_2 in the cells or lumina of the bile ducts.

Following larger doses (3 to 5 c.c. per kilo.) the 48-hour liver (Fig. 1) showed an increase in amount, but no change in ThO_2 distribution. The liver cells showed granules of the same size seen in a 1 c.c. per kilo. dose, but the number of granules present was increased proportionately to the dose given. No evidence of liver damage was seen with these larger doses. One month after an intravenous dose the liver showed a slightly altered appearance. The ThO_2 granules in the liver cells were larger and fewer in number, and gave the appearance of having been formed by the aggregation of several of the granules seen in the 48-hour liver cells. The Kupffer cells were much larger, and were oval or round. The entire cytoplasm was filled with finely granular ThO_2 displacing the nucleus, which stained well, to the periphery of the cell. There was no evidence of leucocytic infiltration, necrosis, fibrosis, fatty change or other damage in any of the cells of the liver. The zonal distribution of the ThO_2 was not altered from the 48-hour picture.

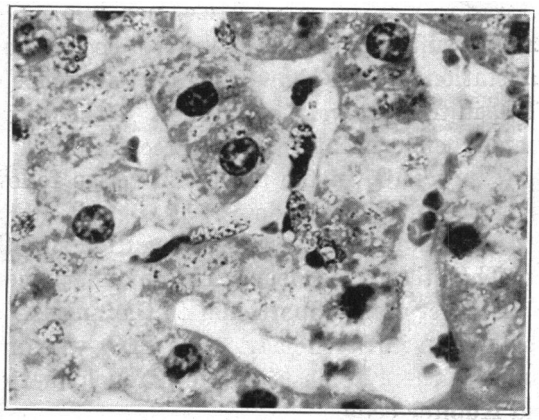

FIG. 1.—High power of liver at 2 days, showing thorium particles within the liver and Kupffer cells.

Two months after the injection of ThO_2 there were definite changes in the thorium distribution. The liver cells contained fewer ThO_2 granules, which were slightly larger than the one month granules and tended to collect about the periphery of the cell. ThO_2-laden Kupffer cells were more numerous, though not changed in appearance or ThO_2-content from the one month appearance. These cells now occupied the middle and inner lobular zones with the exception of a few cells which were found in the region of the portal vein. These Kupffer cells in the inner zone were frequently grouped together in compact aggregations of 5 to 10 cells and only rarely were multinucleated cells seen. No evidence of liver damage was present.

At three months the liver cells contained still fewer ThO_2 granules. The Kupffer cells did not seem to be increased in number and were usually tightly packed around the central vein (Fig. 2). Frequently masses of these cells were seen bulging into the lumen of the central vein. They still maintained, with rare exceptions, cellular individuality, and their nuclei stained well. Occasionally ThO_2-laden cells were seen in the blood of the sublobular veins that were morphologically identical with the ThO_2-Kupffer cells about the central vein.

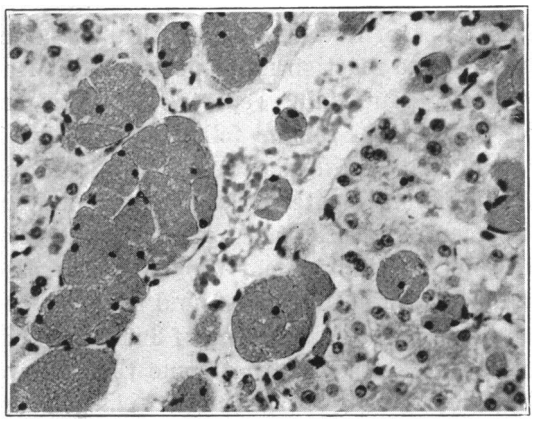

FIG. 2.—Low power of liver at three months, showing thorium-filled Kupffer cells closely aggregated about a central vein area. Two free, thorium-containing cells are seen within the vein lumen.

At four months the liver cells contained still fewer granules. The Kupffer cells had the same distribution as seen at three months, though their number was apparently less. Occasional ThO_2-laden cells were seen in the sublobular veins and had the same morphology as the cells seen in the liver at three months. No evidence of liver damage was present at this period.

Spleen.—The spleen retained relatively large quantities of ThO_2. As in the liver the distribution of the ThO_2 following large doses (5 c.c.

per kilo.) was the same as with smaller doses (0.5 c.c. per kilo.), the difference being only in the amount of ThO_2 present.

At 48 hours many of the cells of the reticulum were bulging with ThO_2 and morphologically resembled the ThO_2 engulfing reticulo-endothelial cells elsewhere. In the periphery and centre of the Malpighian corpuscles many similar ThO_2-laden cells were seen. At this stage much of the ThO_2 lay free in a finely divided state in the reticular spaces. At the end of 2 weeks the free ThO_2 of the reticular spaces had greatly diminished and apparently corresponded to an increase in number and size of the ThO_2-containing reticular cells. These cells were now aggregated and for the most part were fused to form giant cells containing 10 to 25 nuclei. Giant-cell formation was typical of the ThO_2-cells of the spleen, and differed from other tissues where similar cells maintained their individuality. At one month all the free ThO_2 of the reticular spaces had disappeared, apparently engulfed by the reticular cells. The ThO_2 was contained entirely by the reticular cells that for the most part had fused to form giant cells. This appearance did not differ from a spleen examined at 4 months, in ThO_2-content or distribution. Up to a period of 4 months no evidence was seen of any damage to the splenic cells or reaction due to the presence of ThO_2.

Adrenal.—The cells of the adrenal cortex engulfed ThO_2. No ThO_2 was observed in the cells of the medulla. At 2 days the elongated cells lining the blood channels between the columns of the zona fasciculata engulfed most of the ThO_2, but some was present in a very loose arrangement of fine granules in the cytoplasm of columnar cells. As time went on, these cells gradually shifted to the deeper portion of the zona fasciculata and to the zona reticularis. The columnar cells soon lost their ThO_2, which was apparently picked up by the lining cells of the blood channels. At 3 months practically all the ThO_2 was arranged in spherical, eccentric nucleated cells in close relationship to the venous spaces of the outer zone of the medulla (Fig. 3). At 4 months the picture was the same, though the number of ThO_2-laden cells might have been slightly decreased. No evidence of any damage to the adrenal gland by the presence of ThO_2 was seen.

Bone marrow.—The bone marrow was found to contain large amounts of ThO_2 in all cases. Unlike the spleen and lymphatic tissue, the bone marrow ThO_2 apparently did not collect extracellularly in the reticulum, but was found within the reticular cells from the first. The ThO_2-containing cells, morphologically resembled similar cells in the liver and lymphatic glands. These cells did not aggregate in large clumps as in the liver, spleen and lymphatic tissue but remained more or less discrete. The appearance of the bone marrow remained remarkably constant from the first up to a period

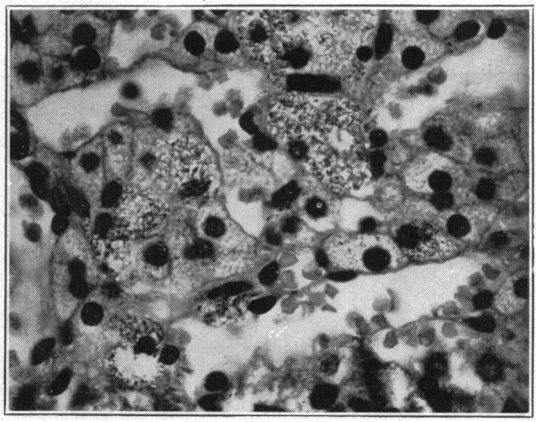

FIG. 3.—High power of junction of adrenal cortex and medulla at three months, showing aggregation of thorium-containing cells about the venous spaces.

of 4 months. The presence of ThO_2 had no effect on the activity of the bone marrow parenchyma, nor did it give rise to any pathological reaction in that tissue.

Lymphatic tissue. — The reticulo-endothelial cells of lymphatic tissue were found to engulf ThO_2 and presented much the same appearance in all parts of the body. The lymphatic glands of axilla, groin, mesentery, and the lymph tissue of the gastrointestinal tract and bronchial tree, all contained ThO_2, in a manner common to all. The ThO_2-engulfing cells were morphologically identical with the ThO_2-containing reticulo-endothelial cells elsewhere, *i.e.*, cytoplasm entirely filled with ThO_2, spherical in shape, and the nucleus round and eccentric.

At 48 hours many small granules of ThO_2 were found lying free in the reticular spaces of the gland, though much of the ThO_2 was engulfed at this stage. At 7 days practically all the free ThO_2 had been engulfed, and the picture presented by the gland at this time was unaltered up to a period of 4 months. The

ThO_2-containing cells were found in the periphery or central part of the lymphatic nodules and also in the reticular portion of the lymphatic tissue. In the lymphatic nodules the ThO_2-cells were usually in compact groups of 5 to 15 cells, usually maintaining cell individuality. In the reticulum the cells occurred singly, or in small groups of 2 to 4. No evidence of ThO_2 elimination was observed up to a period of 4 months. No sign of any pathological process referable to the ThO_2 was observed during that period.

Lung.—Following a dose of 1 c.c. per kilo., the lung, examined at 48 hours, showed occasional alveolar epithelial cells containing

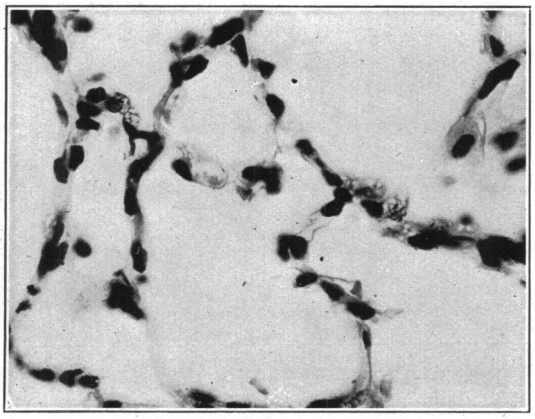

FIG. 4.—High power of lung at 2 days, showing small collections of engulfed thorium in alveolar epithelium.

small amounts of ThO_2 in their cytoplasm (Fig. 4). The amount of ThO_2 present in these cells was not sufficient to obscure the cytoplasm or distort the cell outline. Occasionally these cells were found lying free in the alveolar spaces or in the lumen of the bronchioles. At one month the lung was found to be practically free of ThO_2. From two to four months large, spherical cells, with eccentric nucleus and cytoplasm, entirely filled with ThO_2, were seen, usually singly, in the capillaries of the alveolar walls. These cells bulged the capillary walls and apparently were held *in situ* by virtue of their size, being about 20 μ in diameter. In some cases these cells were seen bulging into or lying free in the alveolar spaces. These cells were seen occasionally in the mucus of the bronchioles and trachea. They were also observed in the blood of the small arteries of the lung. As similar cells were not seen in the veins, it was probable that they were excreted by the lung.

In larger doses (3 to 5 c.c. per kilo.) the 48 hour lung showed the number of alveolar cells containing ThO_2 to be increased, but the amount of ThO_2 in the individual cells was not increased when compared with the picture presented by smaller doses. The spherical ThO_2-laden cells occasionally seen in the blood of the smaller lung arteries were seen in the alveolar capillaries as early as the fourth day. During the first month their numbers increased and they were seen at that time in the alveolar spaces and in the mucus of the bronchi and trachea. There was little change in the ThO_2-

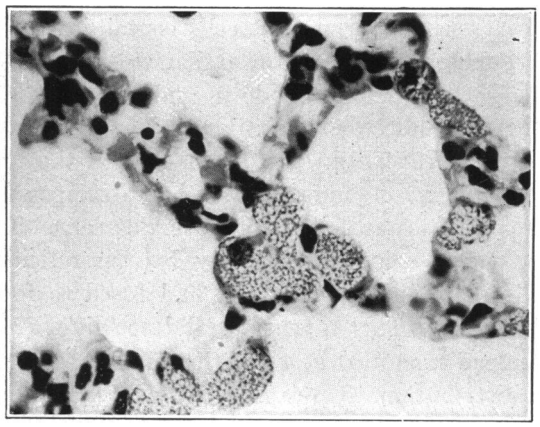

FIG. 5.—High power of lung at two months, showing large thorium-containing cells lodged in alveolar capillaries.

content of the lung at the end of 4 months. (Fig. 5).

No evidence of untoward reaction was observed in the lung at any period that could be attributed to the presence of ThO_2. The ThO_2-cells in the alveolar spaces, bronchi and trachea were occasionally surrounded by polymorphonuclear leucocytes.

Ovary.—Certain cells of the ovary engulfed ThO_2. These cells were present in small numbers and usually occurred singly. The engulfing cells were evidently the reticular cells supporting the lutein cells and in structure resembled the stellate cells of the liver. The amount of ThO_2 present was not sufficient to bulge the cell to any marked degree or displace the nucleus. In some areas the lutein cells evidently engulfed small amounts of ThO_2 in a manner similar to the liver cells. No ThO_2 was seen in any of the cells of the Graafian follicles. The ThO_2 present in the ovary persisted for at

least 4 months and apparently did not change in amount or distribution during such a period. No evidence of any damage due to the presence of ThO_2 was present.

Kidney.—The kidney was found to be almost entirely free of ThO_2. Following some search, a few small granules were usually found in the glomerular tufts or within the tubules of the kidneys of animals up to a period of 2 weeks. These granules were almost without exception extracellular. It was rare to find any ThO_2 in the kidneys after an interval of 2 weeks or more from the time of injection. One exception was found in a kidney where a nephritis was present, a condition not uncommon in laboratory animals. A marked increase in the ThO_2-content was present in extracellular, granular form, principally in the glomerular tufts and tubules. Naegeli and Lauche[13] found a similar concentration in inflammatory areas in lung.

Blood.—Blood smears made less than 5 minutes after ThO_2 injection, failed to show the presence of any flocculated ThO_2. Smears examined after allowing a 10 minute interval showed small particles of flocculated ThO_2. These small particles were found in blood smears 48 hours after injection, but were not seen at later periods. The blood cells did not engulf ThO_2, with the exception of an occasional endothelial cell that contained a few fine granules of ThO_2. Huguenin *et al.*[3] report that in arteriography the vessel shadows fade rapidly after an interval of 5 minutes, which corresponds with the flocculation of the colloidal ThO_2. Apparently there is some relationship between the fading blood vessel shadows and the flocculation of the colloidal thorium dioxide.

Urine.—Rabbits having albumin-free urine prior to thorium dioxide injection invariably maintained an albumin-free condition, following administration.

SUMMARY

A 25 per cent colloidal solution of ThO_2 when injected intravenously into rabbits circulated for about 5 minutes in the colloidal state and then flocculated. The flocculated particles were engulfed, for the most part, by the reticuloendothelial cells of liver, spleen, lymphatic tissue and bone marrow and by the parenchymatous cells of the liver. A moderate amount was lying free in the reticulum of the spleen and lymphatic nodes. A relatively small amount was picked up by the adrenal gland and ovary. The ThO_2 in a finely divided state in the liver and spleen absorbed many of the roentgen rays that usually penetrated these organs, resulting in a shadow which permitted their visualization. The shadow cast by the bone marrow was obscured by the covering bone. In large doses the lymphatic glands could be visualized. The adrenal and ovary contained relatively so little ThO_2 that the dosage required for visualization would necessarily be tremendous and impractical.

The presence of ThO_2 was innocuous in the tissues and no untoward reaction due to its presence has been observed in doses up to 5 c.c. per kilo., during a period of 4 months.

No evidence of elimination of ThO_2 from the spleen, bone marrow, lymphatic glands or ovary has been observed in a period of 4 months. There was evidence that the ThO_2-laden cells of the adrenal gland were passed very slowly into the adrenal vein and probably were later caught in the lung capillaries. The ThO_2 of the liver cells gradually accumulated in the Kupffer cells, which migrated to the central vein area, passed into the blood stream, and through the right heart to the lungs, where they stuck in the capillaries and eventually were cast off in the bronchial mucus.

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REFERENCES

1. BAUMANN AND SCHILLING, *Klin. Wchnschr.*, 1931, **10:** 1249.
2. HUGUENIN, NEMOURS AND ALBOT, *C. R. Seances d. l. Soc. d. Biol.*, 1931, **108:** 879.
3. HUGUENIN AND NEMOURS, *Revue de Pathol. comparée et d'Hygiène générale*, 1931, Nr. 415, s. 480.
4. KADRNKA, *Schweiz. med. Wchnschr.*, 1931, **61:** 425.
5. KADRNKA, *J. de Radiol.*, 1931, **15:** 291.
6. KADRNKA, *Fortschr. Roentgenstr.*, 1931, **44:** 9.
7. KADRNKA AND ROSSIER, *Acta Radiol.*, 1931, **12:** 369.
8. KRYNSKI, *Warszaw. Czas. lek.*, 1931, Nr. 32.
9. LAMBIN, *Comptes rendus Soc. Biol.*, 1931, **108:** 264.
10. LEIPERT, *Wien. klin. Wchnschr.*, 1931, **44:** 1135.
11. MONIZ, PINTO AND LIMA, *Roentgenpr.*, 1932, **4:** 90.
12. MURAMATSU, *Grenzgebiet*, 1931, Nr. 9.
13. NAEGELI AND LAUCHE, *Med. Klin.*, 1931, **27:** 1878.
14. POPPER AND KLEIN, *Mewnch. med. Wchnschr.*, 1931, **78:** 1829.
15. RADT, *Verh. dtsch. Ges. inn. Med.*, 1931, **43:** 443.
16. RADT, *Med. Klin.*, 1930, **26:** 1888-1891.
17. RANDERATH, Institut der Medizinischen Akademie, Düsseldorf, 1931.