diagram are the empirical regression lines. The correlation coefficient is 0.003.

Conclusion. The extremely low correlation found in these experiments suggests that the propulsive motility of small intestine is essentially independent of that of the stomach.

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Received May 22, 1959. P.S.E.B.M., 1959, v101.

Precise Zone of Localization of Anti-Kidney Antibody in Various Organs.* (25025)

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Antibodies prepared against rat kidney, when injected intravenously into rats, are fixed by kidney and several other normal tissues, as shown by radiolabelled antibody technics(1,2). The precise structures on which localization takes place have been visualized by immunohistochemical means in kidney and adrenal(3,4) but not in other organs. Presence of antigens in various tissues, indistinguishable from kidney antigens has been shown. Radiolabelled antibody technics show that antisera against various tissues contained kidney localizing antibodies(1,2); these tissues are capable of absorbing out the localizing activity(5). Baxter and Goodman have shown that antisera to various tissues can produce glomerular lesions and that these tissues can neutralize kidney localizing antibodies(6,7). Cruickshank and Hill(8) demonstrated by immunohistochemical methods the presence of identical antigens in these tissues which react *in vitro* with antibodies formed against rat kidney when cells are sectioned and the antibody brought in contact with cell contents. The fact that cross reacting antigens are found in various organs does not mean that circulating antibodies have access to them. The present work was done to determine just which structures the *in vivo* administered antibody do give a high enough local concentration to be seen by immunohistochemical procedures.

Materials and methods. Rabbit anti-rat kidney serum was that used previously(9). A total of 4 female and 4 male rats weighing 150-200 g were injected intravenously in the tail with 2 ml of rabbit anti-rat kidney serum or normal rabbit serum (heat inactivated for 30 min at 56°C). All animals were sacrificed and perfused 18 hr after intravenous injection of antibody. Uninjected control rats were also used. Fluorescein and tetramethylrhodamine labelled horse anti-rabbit globulin were prepared as previously described(10) by procedures similar to those described by Coons and Kaplan(11). The preparations were absorbed 3 times with rat liver sediment to remove components which stained rat tissues nonspecifically(12). Immunohistochemical staining was performed as follows: Tissues were excised, frozen with solid CO_2 and stored at −20°C. Frozen sections 5 μ thick were cut and thawed on gelatinized slides; dried under a lamp and used on day of preparation. Sections were immersed in saline and the area around the tissue dried. A few drops of fluorescein or tetramethylrhodamine labelled horse anti-rabbit globulin were placed on the tissue and incubated at room temperature 1 hour. The sections were washed 10 min in buffered saline (pH 7) and mounted in glycerol containing 10% buffer. Fluorescence was observed through Reichert fluorescence microscopy equipment. When sections of uninjected rats were stained *in vitro* with rabbit anti-rat kidney antibody prior to treatment with labelled horse anti-rabbit serum, the rabbit antiserum was absorbed with human

* This work was supported in part by U.S.P.H.S. through grant from Nat. Heart Inst.

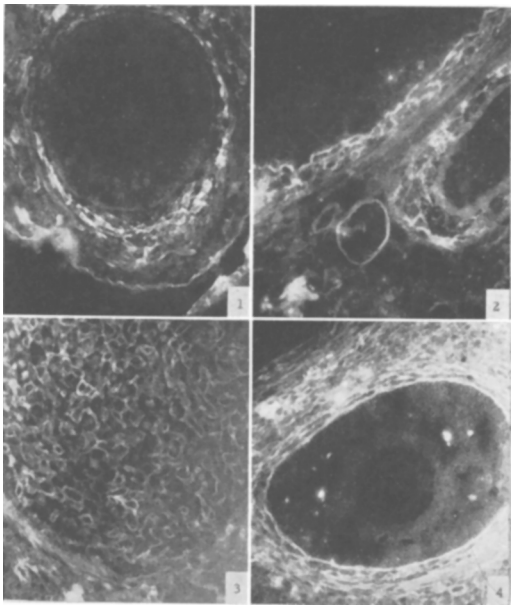

FIG. 1-3. Ovary from a rat inj. intrav. with rabbit anti-kidney serum. Presence of the localized antibody was developed with fluorescein labelled horse antibody to rabbit globulin. Fig. 1 shows staining around follicles and in vascular elements; follicular fluid and follicular cells are unstained. Fig. 2 shows part of a large follicle. Strong fluorescence is seen particularly in inner boundary of follicle. Follicular epithelium is unstained. Vessels and stromal elements are stained, the former somewhat more intensely. Fig. 3 shows staining of an atretic follicle, cellular elements within follicle are unstained but connective tissue elements appeared to be staining strongly indicating that *in vivo* the antibodies have ready access to these areas.

FIG. 4. Ovary section (from normal rat) treated *in vitro* with anti-rat kidney serum and staining developed as before. Major areas of staining are somewhat similar to that seen above. However, all the connective-tissue areas had a greater tendency to stain than is seen in the previous sections indicating that *in vivo* the antibodies did not have as ready access to these areas.

liver sediment to remove nonspecifically staining material. Normal rabbit serum so treated does not stain rat tissues nonspecifically.

Results. When rats were injected with anti-RK serum and assayed for fixed rabbit globulin by immunohistochemical methods, ***kidney, adrenal, ovary*** and ***spleen*** showed structures on which localization took place to a high concentration, ***thyroid, lymph node*** and ***liver*** showed localizations but of lower concentrations, while ***testes, lung, skin, brain*** and ***heart*** showed no staining. When sections of these organs of uninjected animals were stained *in vitro* by direct application of anti-RK serum, staining was essentially that described by Cruickshank and Hill(8). Those organs showing staining following injection of anti-RK serum are described below; figures are shown side by side of *in vivo* and *in vitro* staining.

Kidney and adrenal. In vivo localization of anti-kidney antibody in these organs has been discussed(3,4). Localization was limited to vascular structures and did not involve the multitude of other sites stained when anti-RK was placed directly on kidney sections, as described by Cruickshank and Hill(8). In this latter case, of course antibody is brought

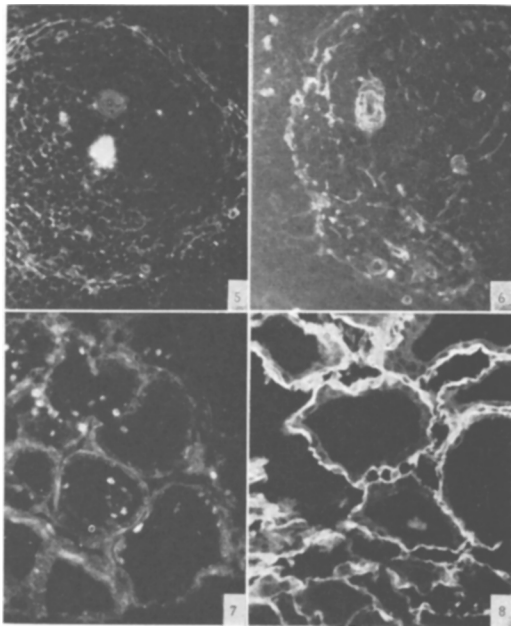

FIG. 5. Spleen from a rat inj. intrav. with anti-rat kidney serum. Fixation of antibody is seen in areas bordering the white pulp. Pulp artery seen in the field is unstained. Bright area directly below pulp artery is an artifact.

FIG. 6. Spleen section treated *in vitro* shows essentially the same type of staining as that described above, however, stroma supporting pulp artery is stained while smooth muscle coat is not.

FIG. 7. Thyroid from a rat inj. intrav. with anti-rat kidney serum. Weak staining is seen just below follicular epithelium while epithelium is unstained. Colloid was washed out in this section, however, when it was retained it remained unstained.

FIG. 8. Thyroid section treated *in vitro*. A similar strong staining is seen in regions corresponding to those in Fig. 7. Colloid from this section was also leached out. Where colloid was retained, it was not stained.

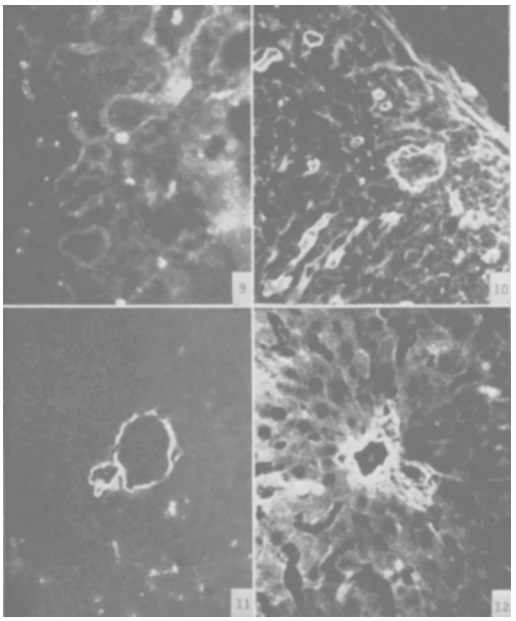

FIG. 9. Lymph node from rat inj. intrav. with anti-kidney serum. Fixation is seen in sub-capsular region as well as in vascular elements. Staining in the vessels is very weak. Capsule and lymphoid cells are unstained.

FIG. 10. Lymph node section treated *in vitro* gives strong staining of the section and is somewhat similar to that seen above, capsule of the spleen, subcapsular region and reticulum of the vessels are fluorescing well. Lymphoids cells are not stained.

FIG. 11. Liver from rat inj. intrav. with anti-kidney serum. Staining is seen in vessels particularly the central veins and in sinusoids radiating from such vascular regions. Sinusoidal staining is very weak and is not apparent in this photograph.

FIG. 12. Liver section treated *in vitro* showing staining of a vessel in the field, stroma surrounding vessel was stained better than *in vivo* section. Sinusoids radiating outward from the vessels are stained, hepatic cells are negative.

directly into contact with intracellular components.

Ovary. (Fig. 1, 2). Strong fluorescence was seen in vascular elements, in stroma surrounding ovarian follicles, and in inner boundary of follicles (region of membrane granulosa and glossy membrane) but the liquor folliculi was negative. In degenerating follicles (Fig. 3), good fluorescence was seen within the follicles. This may be due to the fact that, in the atretic follicle, connective tissue from theca externa penetrates into the lutein mass and forms delicate interlacing septa in which are numerous capillaries which are probably responsible for the observed staining. (Fig. 4 shows *in vitro* staining).

Spleen. (Fig. 5). The capsule of spleen containing elastic fibers and some smooth muscles were unstained. The white pulp stained strongly around the borders, the central artery within the pulp was negative, the red pulp showed some weak areas of staining, the trabeculae were essentially negative. Some macrophages undoubtedly were stained, probably due to phagocytosis of the injected globulin. The other vessels of spleen such as trabecular artery and veins, central artery, pulp artery, and veins, penicillar vessels and venous sinuses were not stained. The strong staining along the periphery of the white pulp may be due to the fact that blood has ready access to this area and reticular fibers and reticular cells are more numerous in this region. (Fig. 6 shows *in vitro* staining).

Thyroid. (Fig. 7). The colloid of the gland did not fluoresce. Neither did the follicular epithelium. Very weak staining was found in the region of reticular connective tissue and capillaries. Due to close proximity of the structures, resolution was difficult. (Fig. 8 shows *in vitro* staining).

Lymph node. (Fig. 9). Specific staining was found under the capsule (subcapsular region), and in elements that appeared to have vascular architecture. Strong staining was seen in polymorphonuclear leucocytes and macrophages. The staining of these and other lymphoid tissue cells may have been nonspecific and due to phagocytosis of the globulin by these cells since similar staining occurred in control sections as well. (Fig. 10 shows *in vitro* staining).

Liver. (Fig. 11) Weak staining was seen in sinusoids surrounding the cords of anastomosing hepatic cells radiating outward from the central veins. The cords themselves were unstained. The strongest intensity was in areas near a central vein. No staining was seen in bile ducts or arteries. The areas which stained are known to be of a reticulum of extremely delicate fibrils, which envelop the sinusoids and a small number of coarser fibers which radiate outward from the region of the central vein. (Fig. 12 shows *in vitro* staining).

Discussion. For an anti-tissue antibody to be cytotoxic for a particular tissue, it must come in contact with the corresponding antigen and be fixed in high enough concentration to cause damage. In the case of injection of anti-rat kidney antisera, there have been many studies showing glomerular damaging effect and there has also been a study indicating malfunction of the adrenal(13). In both of these organs, there have been demonstrated high concentrations of antibody localized on particular structures by means of radiolabelled antibodies(14,15) or by immunohistochemical fluorescein label technics. Localization was found in the glomerular tuft of kidney and the cortical sinusoids of adrenal. In general, immunohistochemical technics have the advantage of greater resolution of the area of fixation if the localized antibody is present at a high local concentration (minimum concentration 28 $\mu g/g$), while radiolabel technics are much more sensitive with respect to diffuse localization(9).

We found that anti-RK localizes to high concentration in certain structures of spleen and ovary and to a much lower concentration on structures in thyroid, lymph node and liver; concentrations in brain, testes, skin, heart and lung were too low to be observed.

Physiological effects might be expected on spleen and ovary and perhaps on thyroid, lymph node and liver on the basis of concentration of localized antibody. Apparently these organs possess sufficient amounts of antigen which can come in contact with the circulation and enough circulation to bring adequate amounts of antibody into contact with the structures to permit them to compete successfully for some antibody with those organs, such as kidney, which are known to trap anti-kidney antibodies very efficiently.

It is of interest to note that while every tissue reported here contained antigens capable of reacting with anti-kidney antibodies, localization of antibodies *in vivo* is not apparent. This must be due to the fact that the antibody does not come in contact with the antigen before it is trapped by the more efficient organs because of the route of blood supply or because the antigens are intracellular and not freely available to sequester circulating antibody.

Summary. *In vivo* localization of antibodies prepared in rabbits against rat kidney was determined by immunohistochemical procedures. Fixation to high concentration was observed in certain structures of ovary and spleen as well as in kidney and adrenal. A lower concentration was observed on structures in thyroid, lymph node and liver, whereas none was seen in testes, lung, skin, brain and heart. Localization of these antibodies *in vivo* is quite different from that observed by treating tissue sections *in vitro* with the antibody. In the latter case, many more structures are stained.

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Received April 13, 1959. P.S.E.B.M., 1959, v101.