reasonably high concentration of Ca^{45} is used, the precision is just as good for ultrafiltrate as for serum, and there is unlikely to be a systematic error in analysis applicable to one and not to the other. As shown by McLean and Hastings(13), the ionic and protein-bound Ca in serum are in dynamic equilibrium, so that the Ca^{45} in serum and ultrafiltrate is a measure of relative values of stable Ca. The point was also checked experimentally by allowing several sera to stand for different lengths of time after addition of the radioisotope. No differences were found. Apparently, therefore, use of Ca^{45} as an analytical tool introduced no systematic error.

Whatever the factors contributing to different values for filtration of Ca and Sr, both ions were affected similarly, and the relatively greater filterability of Sr seems definitely established.

Summary. Sera of patients and normal individuals have been ultrafiltered through cellophane to determine relative filterability of Sr and Ca, using Sr^{85} and Ca^{45} as tracers. In every case, Sr was found to filter more completely, values for 37.5° averaging close to 60% for Sr, and 40% for Ca, calculated on the basis of total serum filtered. None of several variables tested affected relative filterability of the 2 ions. Neither addition of stable Sr nor slight changes in pH, such as might result from manipulation during the experiment, produced significant changes in filterability. There appeared to be a slight negative temperature effect, values for both isotopes being lower at 37.5°C than at 23°C, although the effect on Ca^{45} was usually negligible. Results indicate that Ca is more completely bound to protein than is Sr, more Sr remaining in a free form.

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In vivo Fixation of Antibodies in the Adrenal.* (24212)

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Bale and Spar(1) have shown by radiolabelled antibody technics that antibodies prepared in rabbits against rat kidney, when injected into rats, are fixed in the adrenals. This antibody may be an important factor in disease processes which have been attributed to action of anti-kidney antibodies. Its presence in high concentration in a particular site

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may affect the function of the gland. It is known that the hormones of the adrenal gland can stimulate more than one physiological process. Thus, some conditions observed in experimental glomerulonephritis(2) or experimental toxemia of pregnancy(3) may be attributed to anti-kidney antibodies present in this gland. Indeed, injection of rabbit anti-rat kidney serum has been reported by Lippman to cause an increase in adrenal size (2). However, he found no evidence of tissue damage in the gland on histological examination. The purpose of our report was to determine more precisely the site of the localized antibody in the adrenal gland by immunohistochemical fluorescent stain method. This technic is capable of greater resolution than is possible with radioiodine label methods although the latter are more sensitive(4) and permit detection of smaller amounts of fixed antibody.

Materials and methods. The rabbit anti-rat kidney serum was that used previously (4). The globulin fraction (G-anti-RK) was labelled with I^{131} in accordance with our procedure(5). Free iodide was removed by dialysis against borate buffered saline rather than by the use of an ion exchange resin. Approximately one atom of iodine/molecule of protein was found after iodination. The globulin fraction of horse antiserum against rabbit globulin was that described previously (6). It was coupled with fluorescein isocyanate according to the procedure outlined by Coons and Kaplan(7), or with tetramethylrhodamine isocyanate as described by us(8). Prior to use, labelled horse antibody was treated with wet rat liver sediment to remove nonspecifically staining materials(9). All animals were sacrificed and perfused 18 hours after intravenous injection of antibody. Radioactivity measurements on perfused organs were made in a well-type scintillation counter when required and the tissue was then quickly frozen. Immunohistochemical staining was performed as follows: Tissue sections of thickness 5 μ were cut, placed on slides, and air dried. The slides were immersed in saline, the area around the tissue wiped dry and the sections stained with the fluorescein or rhodamine labelled antibody. Incubation was carried out at room temperature for 1 hr and washing limited to 10 minutes in buffered saline with constant agitation. The tissues were mounted in glycerol and observations made under a Reichert fluorescence microscope.

Results. Concentration of G-anti-RK which must be present in the adrenals to be detected by the fluorescein antibody was determined as follows: Varying quantities of I^{131} labelled G-anti-RK were injected intravenously into rats. Adrenals and kidneys were assayed for radioactivity by counting, and for presence of rabbit antibody globulin by the fluorescein staining technic. Animals injected with radioactive normal globulins (GNS) served as controls. When a dose of 6.9 mg of radiolabelled G-anti-RK was injected into a rat, fixation to the extent of 90 μg and 26 μg protein/g tissue was obtained for the adrenals† and kidneys, respectively. In a rat receiving 15 mg, there was localization to the extent of 115 and 98 μg/g of tissue for these organs. At these concentrations, staining of adrenals was readily observed. A control animal given 15 mg of radiolabelled GNS localized 20 μg/g of adrenals and 15 μg/g of kidneys. However, this was not detected by the fluorescein labelled antibody. The reason for this lack of staining is apparently due to diffuse distribution of the GNS in the organs rather than being localized and concentrated in specific sites as the G-anti-RK.

Two rats receiving 2.2 mg of G-anti-RK localized to the extent of 7.6 to 10.6 μg protein/g of adrenal and 6.8 to 6.9 μg protein/g of kidney by the radioactive assay but positive staining by the fluorescein procedure was observed only in the kidneys. Control animals given approximately the same dose of GNS intravenously showed no staining.

Although the average concentration of localized antibody was greater in the adrenal

† Localization in the adrenal expressed/g tissue must be considered to be a rough estimate because of abberations in weight of such a small organ (10-20 mg) due to perfusion and drying during the weighing process.

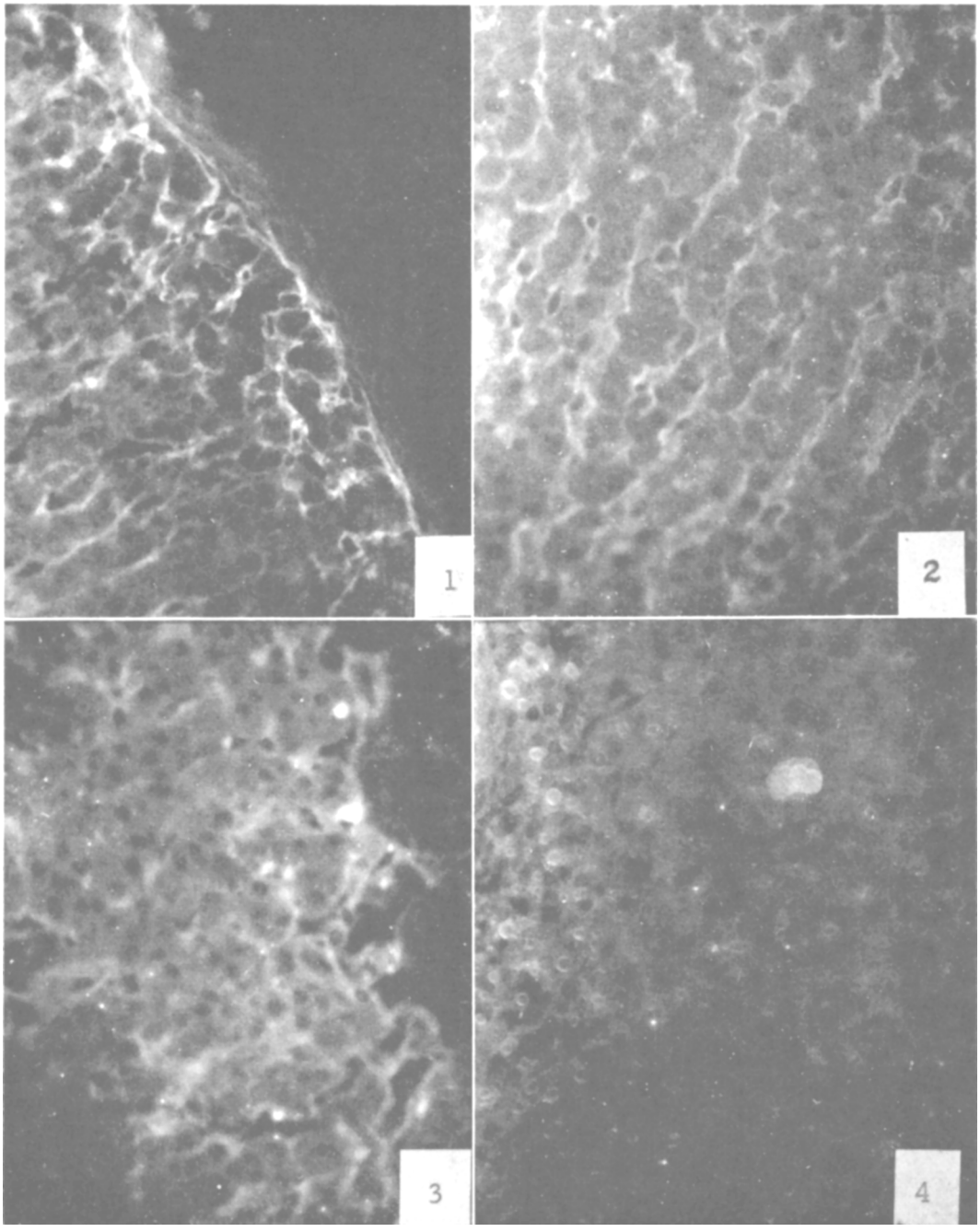

FIG. 1-3. Sections of adrenal from rats inj. with 15 mg G-anti-RK and stained with fluorescein labelled horse anti-rabbit globulin antibody.

FIG. 1. Presence of rabbit anti-rat kidney antibody is seen in the sinusoids of the zona glomerulosa. No fixation in capsule or parenchymal cells seen.

FIG. 2. Fixation is seen in sinusoids of the zona fasciculata.

FIG. 3. Fixation is seen in sinusoids of the zona reticularis up to the medullar boundary. No fixation of G-anti-RK was observed in the medulla.

FIG. 4. Adrenal from rat receiving 15 mg GNS and stained as above. No fixation was demonstrable in this area of the zona fasciculata.

than in the kidney, actual concentration of antibody globulin at site of fixation was less since antibody could be detected only in kidney at the lowest amounts injected.

After initial use of I^{131} labelled protein to determine limits of detection, studies dealing with the description of the histological site of the localized antibody were carried out with unlabelled G-anti-RK. Control rats were injected with the same amount of globulin from pooled normal rabbit sera.

Fixation of antibody takes place just inside the capsule along the sinusoids when small amounts, 4 to 7 mg, of G-anti-RK are administered. When a dosage of 15 mg or greater is administered, the sinusoids throughout the organ show the fixed antibody. This gives the appearance that the antibody is being cleared as it progresses through the adrenal from the capsule inward. The cells near the capsule are more advantageously situated with respect to fresh blood; in these areas fixation seems to take place first with appreciable clearance of antibody from the blood (Fig. 1). Only when these sites become inefficient due to incipient saturation does localization further inside take place.

The fixation was not limited to the zona reticularis alone as reported by Bale and Spar (1) for antibodies prepared against rat adrenal as observed by radioautography. We found that the antibodies studied here (prepared against rat kidney) were seen throughout the cortex, *i.e.*, also in the zona glomerulosa (Fig. 1) and the zona fasciculata (Fig. 2). No fixation was observed in the capsule or the large polygonal parenchymal cells.

An important observation concerns the lack of fixation in the medulla. Staining occurs up to the medullary boundary but no staining is discernible beyond the boundary (Fig. 3). The absence of antibody in the medulla cannot be due to lack of blood vessels since there is a rich capillary network which surrounds the cords and clumps of cells in this region.

The cells lining the capillaries of the smaller vessels in the medulla are endothelial, whereas those lining the sinusoids in the cortex are littoreal cells of the macrophage system, much like those lining the sinusoids of

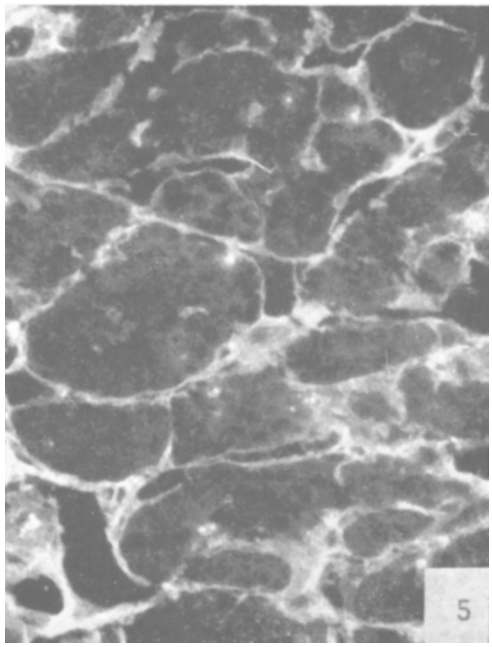

FIG. 5. Adrenal section from rat treated *in vitro* with G-anti-RK and stained as above. Antibody fixed in medulla and in the reticular connective tissue elements surrounding the large parenchymal cells.

the liver and hypophysis (10). This difference in the lining cells may possibly explain the lack of fixation in the medulla of injected anti-kidney antibody.

The medulla does contain substances which can react with anti-kidney antibody when brought into contact with it as when sections of adrenals from uninjected rats are treated with G-anti-RK and developed with fluorescein labelled horse anti-rabbit globulin antibody. In addition to the staining of the sinusoids of the cortex, staining occurs in the capsule and in the reticular elements surrounding the cells of the medulla (Fig. 5). Cruickshank and Hill (11) found similar staining of the adrenal by application of fluorescein labelled G-anti-RK directly. The *in vitro* and *in vivo* situations are different since in the latter case the antibody may not be able to make contact with the antigen.

A similar condition has been shown to exist for the kidney and lung. Anti-kidney antibody is fixed *in vivo* to a detectable concentration only in the glomeruli although it is known to contain antibodies capable of re-

acting with other antigens of the kidney(11, 12). Lung also contains antigens capable of reacting with kidney localizing antibody but does not fix them efficiently *in vivo*(13).

The enlargement of the adrenal gland of rats injected with nephrotoxic serum although showing no histological damage(2) is probably a manifestation of increased permeability of the cortical sinusoids in this organ caused by the extensive localization of the heterologous antibody in these sites.

Summary. The site of fixation in the adrenal gland of rat kidney antibody administered *in vivo* has been demonstrated by the immunohistochemical technic to be along the vessels and sinusoids of the cortex. There is no fixation in the medulla although the medulla does contain antigen capable of reacting with the antibody *in vitro*. The possible derangement of adrenal function may be due to effect of the anti-kidney antibodies.

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Rapid Method of Metabolically Characterizing Individual Tumors.* (24213)

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Characterization of neoplasms as to class by usual methods of pathology is well known to have but limited value in selection of chemotherapeutic agents for treatment of individual tumors. Previous studies have shown that the chromatographic spectrum of metabolic constituents of crude animal tissues often yielded highly specific information allowing for laboratory differentiation of disease processes appearing the same with the light microscope(1). With this in mind a variety of glial tumors together with a number of tumors metastatic to the brain were chromatographed and the patterns obtained after spraying with ninhydrin recorded. In addition to this, fresh tumor tissues were studied individually in relation to amino acids which they took out of a synthetic media of known composition. In this report only the tumors morphologically classed as glioblastoma multiforme will be considered.

Materials and methods. Tumors were obtained in the fresh within a few minutes after surgical extirpation. Representative portions of the tumor were sent to the neuropathology laboratory for the usual pathologic study and diagnosis. Other representative parts were cut into small fragments, using a dissecting microscope to eliminate necrotic tissue as far as possible, squeezed into sheets of Whatman No. 1 chromatographic paper, dried in a hood at room temperature and chromatographed in

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