THE METABOLISM OF FLUORESCEIN-LABELLED AND UNLABELLED EGG-WHITE IN THE RENAL TUBULES OF THE MOUSE

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THE parenteral administration of foreign proteins, of sufficiently low molecular weight to pass through the glomerular membrane, produces a variety of morphological changes in the renal parenchyma. Native egg-white protein is particularly active, as shown by Oliver (1948), and the changes occurring in rat kidney have been very thoroughly described by Oliver, Macdowell and Lee (1954*a*). Until recently there has been no general agreement on the mode of absorption of proteins which appear in the glomerular filtrate. In particular, the " hyaline " droplets which characteristically appear at some stages of resorption have, in the past, been variously interpreted as the original protein, as degenerative phenomena or as secretion droplets of new material. These interpretations are still held by some authorities.

More recently Zollinger (1950) and Rüttimann (1951), using phase contrast microscopy, have described the intracellular granules and droplets in the cells of the proximal convoluted tubules as altered mitochondria. The work of Oliver *et al.* (1954*a*) and Oliver, Moses, Macdowell and Lee (1954*b*), combining morphological studies and conventional histochemical methods, has produced strong support for this thesis, and Oliver, Straus, Kretchner, Lee, Dickerman and Cherot (1955) regard the droplets as a "combination of the excess protein and mitochondrial substances and their enzymes ".

The electron microscope studies of Rhodin (1954) have confirmed the views of Zollinger, Rüttimann and Oliver in showing that, in the mouse, the changes produced by intraperitoneal injection of egg-white are confined to the " mitochondria of the first two-fourths of the proximal convoluted tubules ", but they have not upheld the views of Zollinger on the participation of the microsomes in the formation of granules. According to Rhodin, 7 hours after the injection of egg-white the mitochondria and a finely granular substance coalesce to large granules, while from 18–30 hr. after injection the cell is filled with these granules. Later, restoration of mitochondria takes place, in part from the large granules themselves.

These investigations, even those employing histochemical methods for protein, afford only indirect proof of the incorporation of protein into the droplets. More direct proof is offered by Straus and Oliver (1955) who have found that in kidney homogenates, prepared 18 hr. after the injection of egg-white, the highest content of egg-white by immunological assay is present in the fraction containing the droplets.

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The use of labelled proteins, which might be expected to provide more direct evidence, is derived from the work of Smetana (1947), who first employed coloured azo proteins for this purpose, and from that of Kruse and McMaster (1949) who used γ-globulin azo-coupled to the dye Echt saüre blau. Provided that the protein can be shown to retain its label throughout its existence *in vivo*, methods such as these would seem to offer a solution to the problem. However, from the work of the above authors and that of Gitlin (1950), it is evident that only the broad disposition of the injected protein can be traced. Four further possibilities exist : (1) *Chromogenic labelling* (the attachment to the protein of a small colourless group which is subsequently converted into a highly coloured dye) ; (2) *Fluorescence labelling* (the attachment to the protein of groups conferring the property of fluorescence in ultra-violet light ; and (3) *Fluorescent antibody methods* (the tracing of an antigenic protein by attaching to it *in vitro* its fluorescein-coupled antibody). (4) *Isotope labelling*, followed by auto-radiography.

We have used, unsuccessfully, a method employing the first principle (briefly described in the section on technique) with which interpretation was no easier

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EXPLANATION OF PLATES

All figures represent 5 μ paraffin sections of mouse kidney.

All fluorescence exposures 20 min. unless otherwise indicated.

FIG. 1.—(Freeze-dried) 2 hr. after injection of labelled egg-white (unpurified). Shows specific fluorescence of tubules and "unspecific" fluorescence (not autofluorescence) of elastica. × 800.

FIG. 2.—(Freeze-dried) 4 hr. after injection of purified labelled egg-white (FEF). General view. Shows strong fluorescence of some groups of proximal tubules, weak or absent in others. Exposure 5 min. × 300.

FIG. 3.—(Freeze-dried) 30 min. after FEF. Strong fluorescence in tubular lumen, brush borders and around the tubular nuclei. × 800.

FIG. 4.—(Freeze-dried) 30 min. after FEF. Strong fluorescence of the connective tissues of the renal capsule. × 800.

FIG. 5.—(Freeze-dried) 4 hr. after FEF. Shows strong fluorescence of the brush border and diffusely fluorescent cytoplasm. Tubular lumina and blood vessels are also strongly positive. × 600.

FIG. 6.—(Cold formalin-fixed). As Fig. 5. Localisation of specific fluorescence is identical. Autofluorescence greatly increased. × 600.

FIG. 7.—(Freeze-dried). Antigen-antibody reaction, 2 hr. after injection of F.E. Nuclei and perinuclear rings show strong fluorescence. Brush borders also positive. × 800.

FIG. 8.—(Freeze-dried) 8 hr. after FEF. Strong fluorescence of lumina, brush borders, and in cytoplasm of tubule cells. Exposure 7 min. to show strongest localisations. × 600.

FIG. 9.—(Helly-fixed). As Fig. 8 but exposure 30 min. Shows same localisation of fluorescence but indicates strong quenching effect of Helly fixation. × 600.

FIG. 10.—(Freeze-dried) 8 hr. after FEF. Collecting tubules strongly positive. × 600.

FIG. 11.—(Helly-fixed) 8 hr. after FEF. Perinuclear masses are numerous. Iron haematoxylin. × 800.

FIG. 12.—(Freeze-dried) 18 hr. after FEF. Moderate even fluorescence of cytoplasm and brush border. Nuclei now free. Exposure 15 min. × 800.

FIG. 13.—(Freeze-dried, post-fixed Helly) 18 hr. after FEF. Fine intracellular droplets are present and these are therefore unlikely to be a fixation artefact. Iron haematoxylin. × 800.

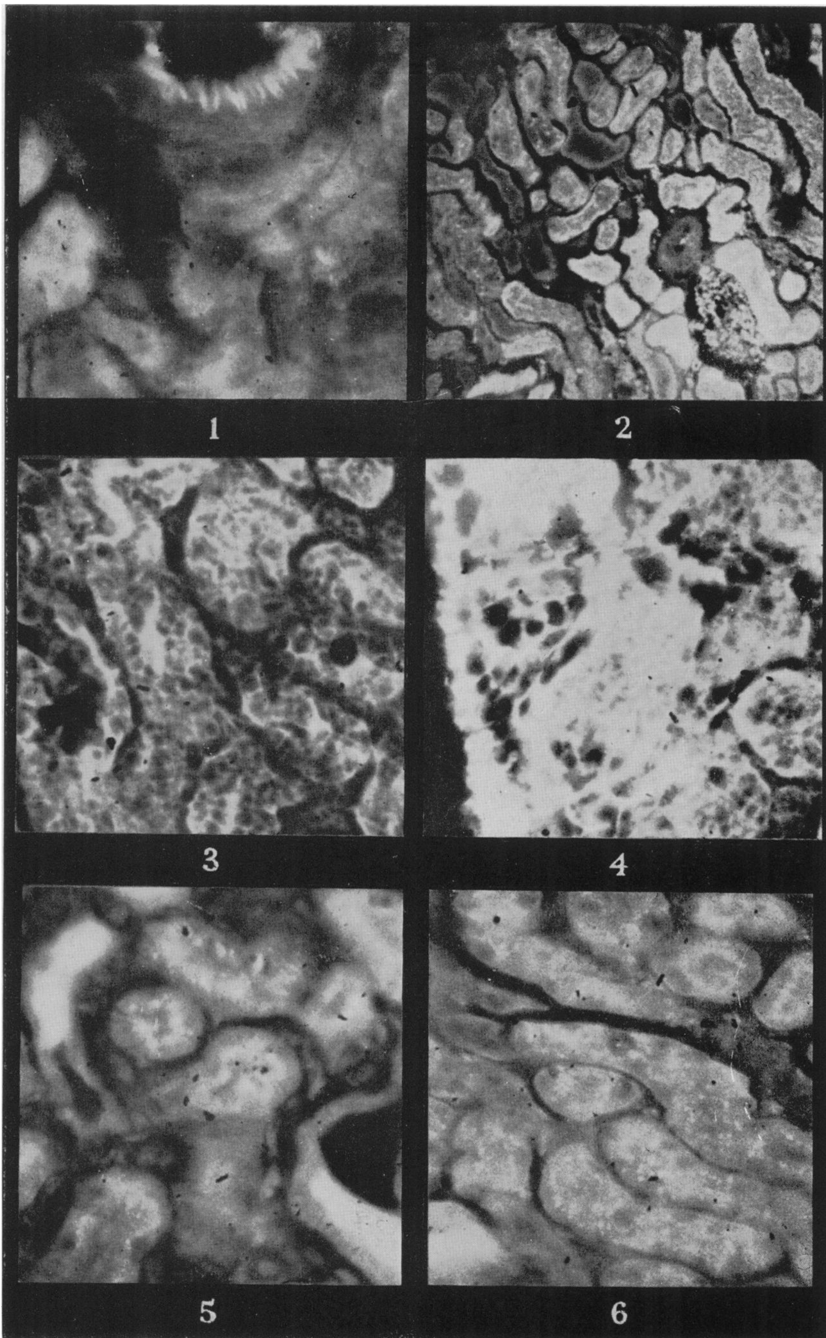
FIG. 14.—(Freeze-dried) 30 hr. after FEF. Fairly strong general fluorescence of the cytoplasm and still visible brush borders. Note strongest fluorescence outlining the basement membranes. × 800.

FIG. 15.—(Helly-fixed) 30 hr. after FEF. Rebuilding of mitochondria is taking place, but perinuclear and nuclear masses and droplets are still present. Iron haematoxylin. × 800.

FIG. 16.—(Freeze-dried) 8 hr. after FEF. Distal tubules free from specific (fluorescein) fluorescence. Blood vessels positive. × 600.

FIG. 17.—(Freeze-dried) 30 hr. after FEF. Distal tubules and Henle's loops show strong specific fluorescence ; blood vessels negative. × 600.

FIG. 18.—(Cold formalin) 8 hr. after FEF. Shows strong "clotting" effect of this fixation. The masses are mainly confluent. Iron haematoxylin. × 800.



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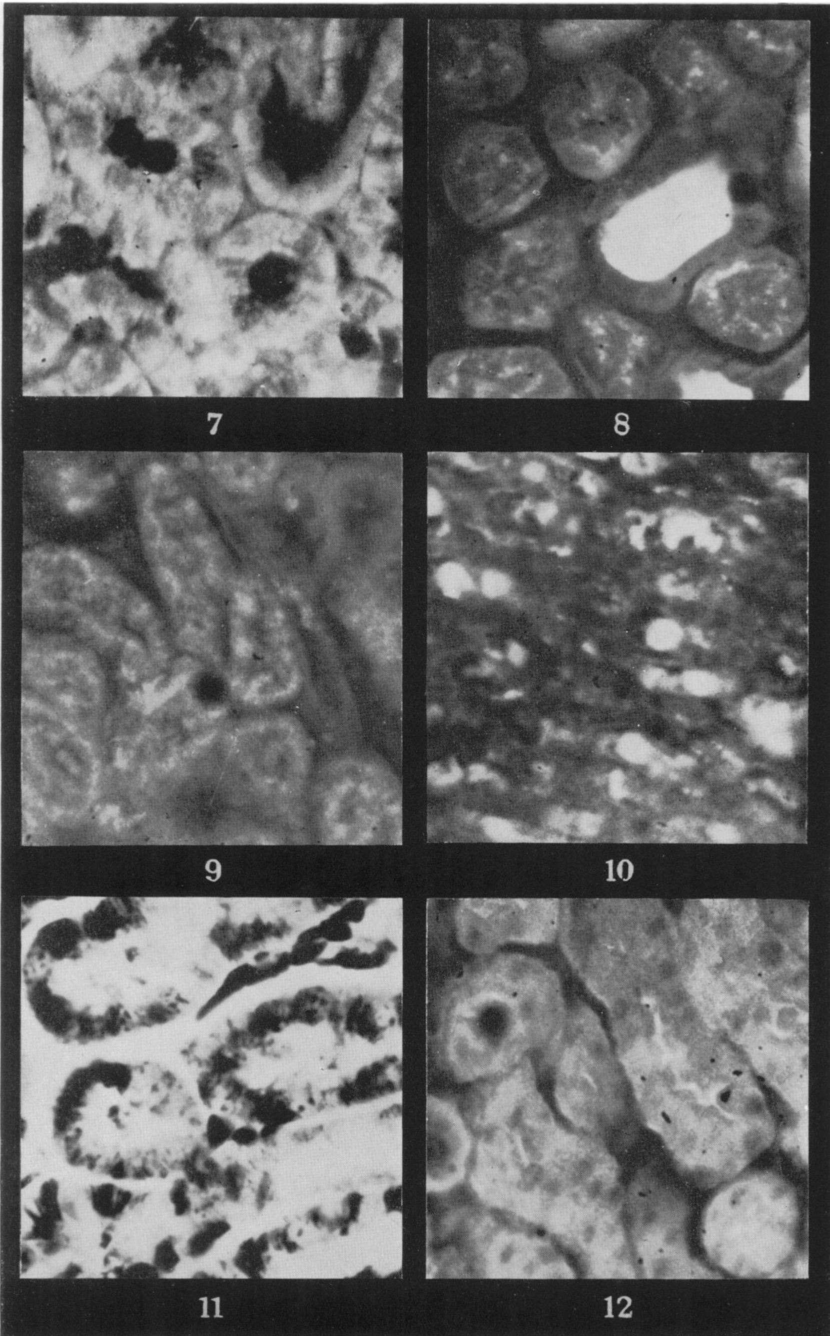
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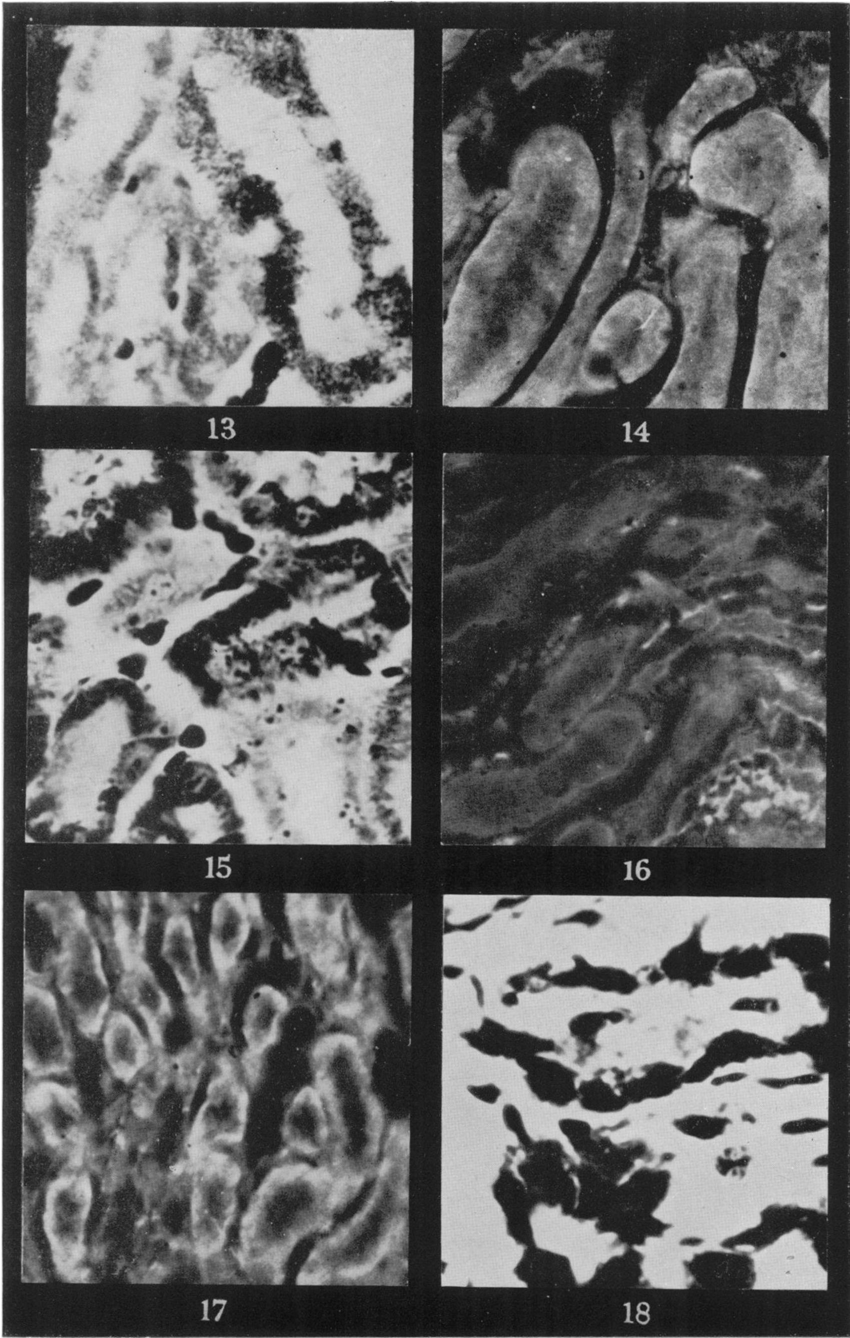

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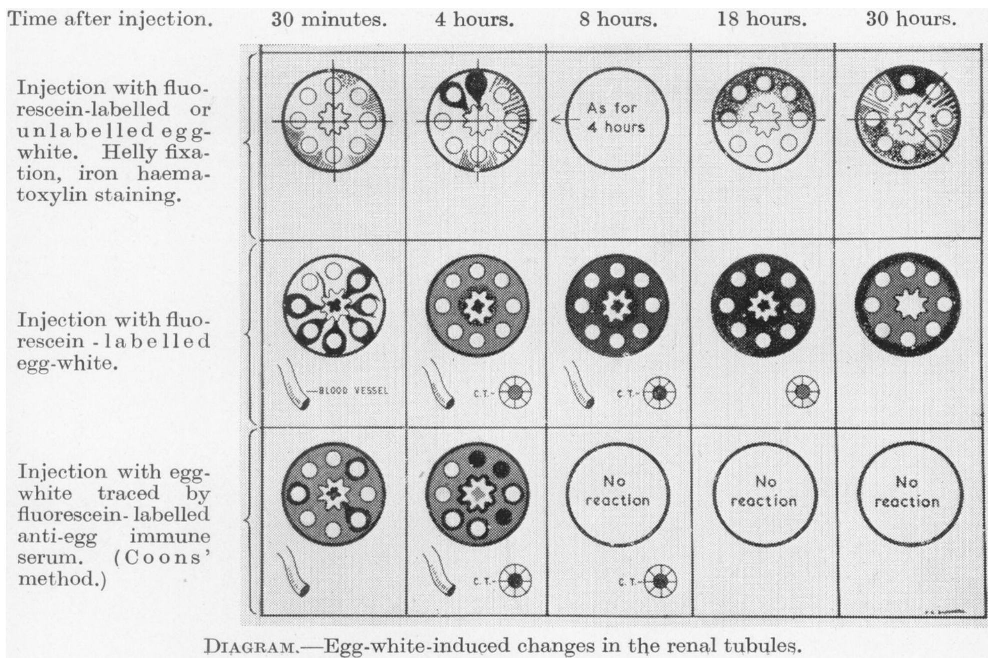

DIAGRAM.—Egg-white-induced changes in the renal tubules.

than with the coloured proteins. The second principle has been employed by Schiller, Schayer and Hess (1952–53), whose critical work has established clearly the physical and immunological properties and the general usefulness of fluorescein-coupled protein markers, and the third by Coons and his associates (Coons, Creech, Jones and Berliner, 1942 ; Coons, Leduc and Kaplan, 1951), who have also shown conclusively that such labelling leaves proteins immunologically intact (Kaplan, Coons and Deane, 1950).

The results obtained by Coons appear to differ entirely from those of workers using non-fluorescence techniques and, to some extent from those of Schiller *et al.*, although the latter did not pursue the question very far. In order to throw some light on these differences we decided to apply the fluorescein marker and the fluorescent antibody techniques in parallel with conventional morphological and histochemical studies.

Studies by the fourth method, which we ourselves did not use, are considered in the discussion at the end of the paper.

MATERIAL AND METHODS

Injection Materials

(1) Fresh egg-white (FE) : Egg-white from fresh hen eggs was mixed with the same volume of physiological saline and filtered through gauze. Protein content 67 mg./ml. (2) Crystallised egg-white (CE) : Egg-white from fresh hen eggs, crystallised by the method of Kekwick and Cannan (1936) was dissolved in physiological saline. Protein content 60 mg./ml. (3) Fluorescein-conjugated egg-white : The stock solutions of FE and CE were coupled with fluorescein isocyanate by the method of Coons and Kaplan (1950) and dialysed at 4° against 0·5 M carbonate-bicarbonate buffered saline (pH 7·4) until the latter was free from fluorescence. Further purification of the fluorescein-conjugated fresh (FEF) and crystallised (CEF) egg-white solutions was carried out with acetone-dried mouse and pig liver powder, the undiluted dialysate being absorbed with liver powder without previous precipitation by ammonium sulphate or acetone. Egg-white solution (1 ml.) was mixed with 100 mg. liver powder, shaken gently at room temp. and centrifuged for 20 min. This procedure was repeated twice and the final supernatant was used for injection.

The injection of the unpurified dialysate (FEF or CEF) resulted in diffuse staining of all tissues with predilection for certain sites, such as the elastica of blood vessels (Fig. 1). No satisfactory explanation was forthcoming for this last result, which was quite distinct from the usual and well-known autofluorescence of elastic tissue. Previous blocking with phenol or naphthols of the specific groups in elastic tissue responsible for its affinity for various dyes (including fluorescein) failed to prevent uptake of the component of unpurified FEF or CEF which was absorbed by liver powder. This suggests at least that the fluorescein is not attached to the elastin in the way suggested by Weiss (1954), that is to say by hydrogen bonding through its phenolic groups.

(4) Naphthaldehyde-conjugated egg-white : This was prepared by coupling 3-hydroxy-2-naphthaldehyde (Weiss, Tsou and Seligman, 1954) with egg-white buffered to pH 10, followed by dialysis against carbonate bicarbonate buffer as above. Freeze-dried sections of kidney and liver containing the labelled egg-white were fixed in ethanol and treated with a diazonium salt at pH 7·4 (Fast Blue B). On chromatographic separation of similarly coupled whole serum the albumen fraction gave a strong black colour after treatment with the diazonium salt, but the maximum colour developed in the tissues was a very pale brownish-red.

Experimental Procedure

Groups of 5–6-week-old white female mice (20–22 g.) were used. These were chosen because recent work by Logothetopoulos and Weinbren (1955) has shown that naturally occurring protein-containing droplets are not found in the renal tubules of mice of this age and sex. The animals received " Research " mouse cubes and tap water *ad lib.* Each animal was given a single intraperitoneal injection of 2 ml. FE or CE or of the equivalent

amount of FEF or CEF (2·2–2·4 ml.). The animals were killed with coal gas 30 min., 2, 4, 8, 12, 18, 20, 24 and 48 hr. and 3, 5 and 10 days after injection. The organs were taken out as quickly as possible and cut into thin slices. Each slice was either immediately quenched in Arcton 6 (I.C.I. Ltd.) as recommended by Bell (1952), cooled to about $-150°$ with liquid nitrogen, and subsequently freeze-dried, or placed in cold 12 per cent buffered formalin or Helly's fixative. After paraffin embedding serial sections were cut at 5 μ, those from freeze-dried material being mounted on clean slides with finger pressure only. The freeze-dried sections were de-waxed in light petroleum and the tissues fixed by 30 min. immersion in absolute ethanol. In a few cases post-fixation of the freeze-dried sections was done with Helly.

Light microscopy.—For light microscopy sections prepared by each of the above methods were stained by Heidenhain's iron haematoxylin, Gram's method, methyl green/pyronin (Trevan and Sharrock, 1951), a trichrome periodic acid-Schiff method, and by Schiff's reagent after oxidation with aqueous periodic acid. Some sections were treated before staining with crystalline ribonuclease (0·5 mg./10 ml. 0·1 M veronal acetate buffer at pH 8·5). Buffer was used in order to obtain results comparable with those of Oliver *et al.* (1954*b*).

Coons' method.—For the tracing of injected FE and CE the method of Coons and Kaplan (1950) was employed. Anti-egg-white immune serum was obtained by injecting a rabbit with 2 × 2 ml. FE and 2 × 2 ml. CE four times weekly for 6 weeks. One week after the last injection the animal was bled (serum titre 2560). A rough globulin fraction obtained by half saturation with ammonium sulphate was conjugated with fluorescein isocyanate (2 mg. isocyanate, calculated as amine, in acetone per 100 mg. protein) and dialysed and purified in the same way as the fluorescent injection material. Both were stored at $-20°$. Only freeze-dried ethanol-precipitated sections were used for the antigen-antibody reactions.

Fluorescence microscopy and photomicrography.—A standard monocular microscope with a simple glass condenser was used. The light source was a 350 watt mercury vapour lamp (B.T.H. Mercra) with Wood's glass filter and a 20 mm. cuvette filled with aqueous copper sulphate. In the eye-piece 4 thicknesses of Wratten 2B gelatin filter were placed in order to cut out U-V radiation entirely.

For photography the same apparatus was used. The most suitable film was found to be Kodak Fluorodak green-sensitive. This requires a short exposure time (10–20 min.) but has unfortunately a rather large grain size so that enlargements greater than × 3 or 4 are not possible. With fine-grain films (Kodak Microfile) the exposure time was > 3 hr.

The sensitivity of Fluorodak permitted registration of the smallest amounts of yellowish-green (fluorescein) fluorescence while recording the blueish-white autofluorescence of the tissues only weakly. In the fluorescence photomicrographs which illustrate this paper the exposure times (except where specially noted) are identical; differences in intensity of the emitted light are therefore demonstrated.

RESULTS

(The results are illustrated schematically in the accompanying Diagram)

Fluorescent-labelled Protein Injection and Fluorescence Microscopy

The description of our results refers mainly to freeze-dried sections, but it was observed that the localisation of the fluorescent marker was independent of fixation, being the same in cold formalin and Helly-fixed preparations. Cold formalin fixation, however, increased the amount of bluish autofluorescence without affecting fluorescein fluorescence, while Helly fixation had a strong quenching effect on both. Comparison between Fig. 5 and 6, and between Fig. 8 and 9, should emphasise these points. Freeze-dried de-paraffinised sections are ideal for fluorescence microscopy. The primary (auto-) fluorescence of the tissue corresponds to that given by fresh unfixed frozen sections.

After injection of FEF or CEF, a suitable short time interval showed the fluorescent marker strongly concentrated by a proportion of nephrons in the renal cortex; others contained small amounts or none (Fig. 2). With increasing

time after injection a higher and higher proportion of nephrons contained the dye until after 24 hr. practically all the proximal convoluted tubules emitted a low but even greenish fluorescence.

We found strong fluorescence in the lumina of the tubules (of positive nephrons) 30 min. after injection and at the same time the brush borders became positive. Within the cell fine linear fluorescence surrounded the nuclei (see first column, 2nd row, in the Diagram) and in a photomicrograph (Fig. 3) this presents as a net-like appearance because the autofluorescence of the cytoplasm is too weak to form an image. The only other positive elements at this time are the blood vessels and the connective tissue of the renal capsule (Fig. 4). These two sites continue to be positive through the two following phases (4 and 8 hr.). After 4 hr. localisation of the marker is largely the same as in the previous phase, but the lumen contents and blood vessels become very bright. In the brush borders there is a strongly fluorescent material which is often seen in droplet form. In the photomicrographs (Fig. 5 and 6) there appear to be droplets also in the cytoplasm, but these are really situated on the surface of the lining cells.

Between 4 and 8 hr. (compare Fig. 5 and 8) no difference in localisation of the marker could be seen, but in all situations fluorescence was much brighter. For this reason, and to show clearly the strong fluorescence of brush borders and lumina, Fig. 8 was taken with a short exposure time. At this time (8 hr.) the distal tubules and Henle's loops are non-fluorescent (Fig. 16), but the contents of the collecting tubules are strongly demonstrated (Fig. 10). Their cytoplasm shows only autofluorescence.

After 18 hr. fluorescence in the injected kidney is diminishing. Nearly all the proximal convoluted tubules emit an even green light (Fig. 12) and the brush borders are distinctly less obvious. The content of the lumina is much decreased in fluorescence.

The cytoplasm of the proximal convoluted tubules continues to emit the same amount of light 30-48 hr. after injection, but the basement membranes are now strongly outlined (Fig. 14). Visible to the eye, but not shown in the photomicrograph, there now appears a fine striation in the basal parts of the cells as if the regenerating mitochondria (Fig. 15) were displacing the marker-containing cytoplasm. In the brush border zone, in the tubular lumina and in the blood vessels no fluorescence could be observed, but in the cytoplasm of the distal convoluted tubules and Henle's loops we now observed a relatively strong even fluorescence with occasional bright granules. Fig. 16 shows the distal tubules, 8 hr. after FEF, free from fluorescent marker though the blood vessels are strongly positive. Fig. 17 shows distal tubules and Henle's loops 30 hr. after FEF. The comparison is quite striking.

Three days after injection fluorescence remains qualitatively similar but quantitatively much less, while after 10 days the tubules are indistinguishable from those of controls except that there is an increase in the autofluorescence of their cytoplasm. Occasional phagocytes in the capsular connective tissue show a greenish granular fluorescence which distinguishes the injected kidneys as a whole from uninjected controls.

We observed that ribonuclease produced no change in the disposition or intensity of fluorescence in fixed tissues although some loss occurred with freeze-dried sections. Inactivated ribonuclease and incubation with water alone caused similar changes in unfixed sections. The negative effect of ribonuclease on the

fluorescent marker of fixed tissues is in sharp contrast to its effects in reversing the positive staining of the various masses and droplets with iron haematoxylin (*vide infra*).

Non-fluorescent Protein Injection. Tracing by Coons' Method

Our results with this method confirm those of Coons *et al.* (1951), but differ very greatly from those described above in animals injected with fluorescein-conjugated protein. Most impressive is the fact that the protein can only be satisfactorily demonstrated up to the 2nd hr. or, at most, in the finest traces, up to the 4th hr. The content of the tubules is an exception ; it was reasonably well demonstrated up to the 8th hr. Up to 1 hr. localisation of the fluorescent antibody is mainly in the blood vessels (plasma), in the lumina of the proximal convoluted tubules and in their lining cells. In the latter the first fluorescence to appear takes the form of a ring around the nuclei, shortly afterwards (2 hr.) this has spread diffusely through the cytoplasm. The most remarkable appearance at this time and subsequently is the fluorescence of the nuclei. Fig. 7 shows the appearances of some of the tubules 2 hr. after injection of FE. Here the cytoplasm is mainly diffusely stained and unstained nuclei appear dark ; ringing of the nuclei is apparent and, at the lower right and central border of the photomicrograph there are fluorescent nuclei. No such picture was seen using fluorescein-conjugated egg-white injections.

The content of the collecting tubules became weakly positive with the fluorescent antibody after 2 hr. and it was strongly positive at 4 and 8 hr., in the last instance at a time when no other constituents of the kidney gave a positive reaction.

The specificity of the reaction was controlled by using sections covered first with the unconjugated homologous antiserum (for 30 min.) and then, after washing, with the fluorescent antiserum. No secondary fluorescence was observed in these sections. As a further control, freeze-dried kidney sections from untreated animals were covered with the fluorescent antiserum. Again no secondary fluorescence was observed.

Fluorescent and Non-fluorescent Protein Injections. Conventional Histochemical Methods

The results of applying the iron haematoxylin method to Helly-fixed sections at various intervals after protein injection are illustrated in Fig. 11, 13 and 15 in order to provide comparison with the fluorescence pictures at similar intervals. It is sufficient to state here that our results differ from those of Oliver *et al.* (1954*a*) only in our finding of significant morphological changes in the cells of the proximal convoluted tubules at an earlier stage.

After treatment of the sections with crystalline ribonuclease (6 hr., 37°) we observed that it was almost impossible to stain the mitochondria, the droplets, or the large masses of material (Fig. 11 and 18) by iron haematoxylin, Gram's method, or pyronin. A few droplets occasionally were weakly stained. The Gram-positivity of the droplets was found by Oliver *et al.* (1954*b*) to be " reduced if not entirely removed " by ribonuclease. In this respect agreement is complete ; with the PAS method, however, we noted that the normal strong staining of the

brush borders of the proximal convoluted tubules was unaltered by egg-white injection while the colour of the basement membranes was increased. The droplets were usually only weakly PAS-positive with an occasional more strongly stained example. These findings are not in complete agreement with those of Oliver *et al.* (1954*b*) who suggested that the PAS reaction could easily demonstrate the difference between egg-white-induced and ovalbumin-induced droplets by the significantly higher carbohydrate content of the former.

Studies were carried out on both cold formalin-fixed and freeze-dried acetone-fixed kidneys in order to establish the relationship between the droplets and the strong intracellular non-specific esterase of the tubule cells. Using the indoxyl method of Holt and Withers (1952), we found that although esterase was present in the tubule cells containing the droplets, it was distributed diffusely throughout the cytoplasm. Other histochemical methods, particularly those for protein end groups, showed a very striking similarity between the natural droplets which occur in the rat (see below) and " unnatural " droplets produced by protein injection.

DISCUSSION

The results of the investigations presented pictorially in the second and third rows of the Diagram permit three main conclusions. First, that at all stages of protein absorption by the tubules the highest concentration of immunologically intact egg-white is found elsewhere than in the form of intracellular droplets (which in any case are not observed by the light or electron microscope much before 12 hr.). Second, that the fluorescein marker does not appear in the droplets at a stage when these can be demonstrated morphologically, and that therefore the protein moiety to which the marker remains attached is not concentrated in such a site. Third, that the immunologically intact protein is broken down extremely rapidly to fractions which no longer react with the specific antibody.

Minor conclusions are (1) that the foreign protein-induced droplets demonstrable by conventional histochemical staining methods are strikingly dependent on fixation, whereas the pattern of fluorescent protein is largely independent (Fig. 6 and 18) ; (2) that the esterase-containing predominantly protein droplets studied by Pearse (1953 ; 1954), which are a normal tubular phenomenon in older male rats and a less frequent one in older mice, are very similar histochemically to the egg-white-induced droplets except that the latter lack esterase ; (3) that the metabolism of protein takes place not only in the proximal convoluted tubules as recorded by numerous authorities, but also (Fig. 17) in the distal tubules and loops of Henle. Whether this represents absorption from the tubules or excretion into them is unknown.

We are in complete agreement with Coons in observing the deposition of egg-white traced by fluorescent antibody on and within the nuclei, an observation which is supported by the evidence of Crampton and Haurowitz (1950) that ^{131}I-labelled ovalbumin and bovine γ-globulin can be found in the nuclear fraction of homogenates of rabbit liver. We also agree with Schiller *et al.* (1952–53) that the fluorescein of the fluorescein-labelled ovalbumin fails to appear in the nuclei, although it appears in or on the nuclear membrane. An explanation suggested by these last authors is that the differential permeability of the nuclear membrane excludes fluorescein-conjugated protein. With this hypothesis we agree also.

The probable course of events in the tubular absorption of egg-white, suggested by the results of morphological, histochemical, fluorescent antibody and fluorescent marker studies, is that this protein is absorbed mainly by the brush border zone of the proximal convoluted tubules, through which it passes rapidly into the cytoplasm and thence through the nuclear membrane into the nuclear sap. The intracellular and intranuclear protein is rapidly split, so that the local concentration of immunologically definable products soon falls below the threshold at which it can be demonstrated by Coons' method. The split products remain in the cell at least up to the third day and appear to leave it by passing through the basement membrane of the tubule, and presumably through the adjacent capillary endothelium into the blood stream.

In view of the immunological work of Straus and Oliver (1955), on a purified fraction of whole kidney homogenate which contained only the droplets, the fact that in our hands these never contained either histo-immunologically demonstrable egg-white or fluorescein-marked egg-white is particularly hard to understand. These authors showed that the droplet fraction of their homogenate contained four times more egg-white than any other cellular fraction (excluding the supernatant), 6 per cent of the total protein of the droplets being reckoned as egg-white. It is unlikely that the threshold of Coons' method is above this figure. No information is available concerning the amounts of protein (antigen) demonstrable by the method, although Coons, Leduc and Connolly (1955) state that the amount of protein (antibody) demonstrable by their double technique (antibody-antigen-fluorescent antibody) is " evidently high in concentration and very small in absolute amount ".

For the fact that the fluorescent marker is not seen in the droplets there are two alternative explanations. One is that the droplets are fluorescent but that the diffuse fluorescence of the entire cytoplasm masks the slightly greater intensity of the light given off by the droplets (fluorescent light is emitted in all directions from its source). The second involves some consideration of the possible differences between unmarked and fluorescein-conjugated protein, in order to explain the lack of fluorescent material in the droplets. Although Schiller *et al.* attribute the absence of nuclear " staining " to the barrier effect of the nuclear membrane, they offer strong evidence that in other, and especially immunological, respects no differences exist between the marked and unmarked protein. We cannot exclude the operation of similar barrier effects in the membrane of the mitochondria which prevent absorption of the labelled protein or protein product.

Little is known of the rate of breakdown of protein within the renal cells, although Spector (1954) was inclined to attribute the uncombined (non-protein bound) ^{131}I of the supernatant fraction of his rat kidney homogenates to such a mechanism. It is of interest that he found that " practically no ^{131}I was demonstrable in the cell nuclei ", but this was 17 hours after the injection of protein. Spector found a tenfold increase in protein-bound ^{131}I in the mitochondrial fraction of nephrotic by comparison with normal rats. This evidence supports the immunological findings of Straus and Oliver (1955) referred to above.

On the whole, the work reported in this paper suggests that positive results, using Coons' method or fluorescein-labelled protein injections, can be regarded with confidence. Negative results, particularly if contrary to those of other acceptable techniques, should be interpreted with caution.

SUMMARY

A study of the tubular absorption of foreign protein and the intracellular development of specific droplets is reported. Young female mice were given single intra-peritoneal injections of egg-white, either unlabelled or fluorescein-labelled, and the kidneys, freeze-dried or fixed by conventional methods, were examined by three different techniques.

Two of these (tracing by fluorescent marker and tracing by fluorescent antibody) showed good agreement with each other but little agreement with the third (tracing by conventional staining or histochemical techniques for protein).

It is concluded that egg-white, passing through the glomeruli, is rapidly absorbed by the proximal convoluted tubules. It is equally rapidly broken down to smaller products and ultimately these return *viâ* the basement membrane to the blood-stream.

In the later stages of absorption the fluorescent label appears in the cytoplasm of the distal convoluted tubules. This may indicate uptake from the lumen or from the interstitial tissues.

By neither of the fluorescent methods employed could positive evidence for the presence of whole egg-white in the droplets be obtained.

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