ther DPN or TPN was present in the assay system. The five TDH activities detected did not exhibit the ordered electrophoretic mobilities characteristic of a five-membered set. From the above it seems probable that at least some of the TDH bands detected may have distinct cell localization or metabolic functions, or both.

The present experiments clearly demonstrate the presence of multiple forms of TDH in extracts of certain tissues of the turtle, perch, and trout, as well as in spinach and yeast. With the exception of spinach, all of the organisms exhibiting multiplicity contain at least one five-membered TDH set. Such five-membered sets probably result from the random combination of two homologous, yet distinct, subunits into tetrameric molecules. It is now known that rabbit muscle TDH is a tetrameric molecule composed of identical subunits (*12*). Moreover, the extensive comparative studies of Allison and Kaplan (*13*), as well as the studies of Perham and Harris (*14*), suggest that the TDH molecules from widely divergent sources are closely homologous; hence, it can be inferred that the TDH variants detected here are tetramers. The five-membered sets of lactic dehydrogenase (*15*) and of aldolase (*3*) are the result of random formation of tetramers from subunits which have different amino acid sequences and which are, therefore, coded by separate genes. By analogy, it appears likely that some of the multiple forms detected in the present study will have a similar explanation. Indeed, all of the TDH variants detected could be the result of the synthesis of different TDH subunits, some of which could interact to form hybrids. However, other possibilities for the formation of different subunit types should also be considered. For example, the same polypeptide chain may exist in two or several conformations (*16*); alternatively, a single polypeptide chain may be modified by degradation or by derivative formation. We think it is unlikely that there are separate genes for every TDH variant detected.

The profile of TDH variants in perch and trout tissues are also of interest from a physiological point of view. Although the tissues of both organisms exhibited a characteristic pattern, there is no consistent relationship between the profiles of the same tissues from the two species. Thus, there is no evidence from these studies of an organ-specific distribution and, hence, no apparent specific requirement by an organ for a given variant or set of variants. It will be of considerable interest to determine whether the kinetic properties of the various TDH variants are modulated in accordance with the metabolic activities of the various tissues.

Because of the multiple forms of TDH found in some organisms, and the apparent absence of variants in others, this enzyme may be a useful phylogenetic and physiological probe.

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7. Namely, 0.05*M* imidazole, *p*H 6.5 (90 minutes, 4°C); 0.02*M* tris, *p*H 8.0 (150 minutes, 4°C); 0.02*M* glycine, 0.001*M* ethylenediaminetetraacetate (EDTA), *p*H 10.5 (150 minutes, 4°C); 0.02*M* glycine, 0.001*M* DPN, *p*H 10.5 (150 minutes, 4°C); 0.02*M* phosphate, *p*H 7.5 (90 minutes, 4°C); 0.05*M* veronal, *p*H 8.6 (150 minutes, 37°C); 0.02*M* and 0.05*M* tris, *p*H 8.0 (150 minutes, 37°C). We included 0.07 percent β-mercaptoethanol in each buffer. Tissues were always homogenized in the appropriate buffer; in some cases crystalline horse liver alcohol dehydrogenase was added as a standard for determination of relative mobilities.
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Heterophile Reactive Antigen in Infectious Mononucleosis

Abstract. *Specific heterophile reactive antigen has been localized by means of indirect immunofluorescence in 12 of 13 kidney biopsy specimens obtained during the acute phase of infectious mononucleosis. I feel that this may represent the identification of infectious agent antigen. Evidence is also presented for the possible existence of two different strains of the agent of infectious mononucleosis.*

The etiologic agent of infectious mononucleosis has long eluded detection. Its epidemiology suggests that an infectious agent is responsible and the general suspicion is that the agent is a virus. A rise in titer of heterophile antibody with specificity for beef red blood cells is a curious and specific laboratory finding during the early stages of infectious mononucleosis. Because many viruses have the ability to grow well in tissue cultures of kidney cells (*1*), it appeared feasible to use specific heterophile antibody to search for antigen of the infectious agent in kidney tissue of patients during the acute phase of the illness.

Thirteen patients with typical clinical symptoms and signs of infectious mononucleosis and high serum heterophile titers specific for the antigen of beef red blood cells were investigated. Throat cultures were taken initially and every other day to insure the absence of streptococci. Antistreptolysin O and antistreptococcal F (*2*) titers were also determined initially and at weekly intervals (see Table 1). Urinalyses and urine cultures were made initially and at weekly intervals to help rule out the possibility of any bacterial infections of the genitourinary tract. Double needle biopsies (*3*) of the kidneys were performed on all 13 pa-

tients within 6 to 14 days after the clinical onset of their illness. One piece of tissue was fixed in 10 percent buffered formalin and used for routine histology with hematoxylin and eosin and periodic-acid-Schiff stains. The other piece of tissue was placed immediately in 95 percent ethanol at 4°C and prepared for immunofluorescence as described by Sainte-Marie (*4*).

Heterophile antibody was prepared from each patient's serum (*5*) by means of diethylaminoethyl-cellulose ion exchange chromatography (*6*). The typical elution position of heterophile antibody was consistent with that of gamma M immunoglobulin. Further purification and characterization of the heterophile antibodies was carried out by passage through Sephadex G 200 columns (*7*). All the heterophile antibody activity was present in the first protein peak eluted from the columns, and when these fractions were analyzed in the model E analytical ultracentrifuge, only a single peak with a sedimentation coefficient of 19*S* was observed.

Indirect immunofluorescence studies of the kidney tissues were performed according to the method of Peters and Coons (*8*), with purified heterophile-positive fractions as intermediate layers. Fluorescent goat or rabbit anti-human gamma globulin, purified on diethylaminoethyl-cellulose (*9*), was used to determine whether any specific gamma M globulin localization had occurred. Both fluorescent conjugates produced fluorescent precipitin arcs in agar gel diffusion and immunoelectrophoresis with human gamma G and gamma M immunoglobulins. Control steps used in the immunofluorescence staining procedure were similar to those outlined by Peters and Alper (*10*). Specimens of control kidney tissue were available from patients with acute and chronic pyelonephritis, nephrosclerosis, acute primary atypical pneumonia, and acute lobar pneumonia due to pneumococci. Control conjugates used were fluorescent, polyvalent goat anti-streptococcal and rabbit anti-pneumococcal serums.

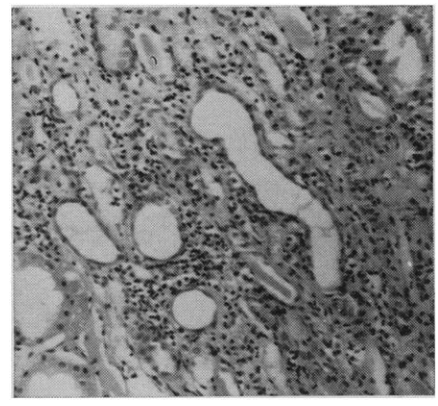

Fig. 1. Kidney biopsy from patient No. 3 on 12th day of acute illness. Note interstitial edema with moderate monouclear cellular infiltration. There is some tubular dilatation with atrophy of lining cells. Some of the tubules contain hyalin casts. The tissue was stained with hematoxylin and eosin. (× 65)

Renal function studies performed on all patients consisted of inulin and radioactive hippuran clearances and maximal tubular reabsorption of glucose (glucose Tm) (*11*). All test results were completely normal, as were all urine cultures. Three patients had mild pyuria (10 to 15 white blood cells per high-powered field in a fresh, unspun, morning urine) intermittently during the first 2 weeks of their illnesses. The pyuria subsequently cleared in all three patients.

Histological examination of the kidney biopsies revealed that 12 of 13 had mild, diffuse, pathological changes. These consisted of glomerular swelling without hypercellularity, patchy areas of interstitial infiltration with mononuclear cells (see Fig. 1), and many clumps of cells within tubular lumens.

Specific fluorescence was detected in the glomeruli and tubular cells in the same 12 patients who demonstrated inflammatory changes on routine histology. One biopsy specimen was interpreted as being completely normal and was also completely negative for any specific fluorescent staining. Figure 2 depicts the type of specific glomerular fluorescence observed, which in all cases was focal in nature. Some glomeruli were completely negative and no glomerulus demonstrated diffuse fluorescence. The specific staining appeared to be intracytoplasmic; however, without electron microscopy precise localization was impossible. Figure 3 illustrates typical fluorescent staining within the cytoplasm of tubular cells. It was diffuse and had a granular appearance. Large areas of tubules gave positive, specific fluorescent staining, whereas other large areas were completely negative. The cytoplasm of many of the cells lying within the tubular lumens also gave positive, specific fluorescent staining. Interstitial tissue did not demonstrate fluorescence and only an occasional tubular cell nucleus looked positive for specific fluorescent staining.

Specific fluorescence in all cases was completely prevented by preabsorbing

Table 1. Serological results on day-0 and day-14 serum samples. All values are reciprocals of the dilutions. Abbreviations: RBC, red blood cells; ASO, antistreptolysin O titers; AS, antistreptococcal antigen, as prepared by D. Fuccillo. Normal heterophile is zero; normal ASO is zero to 128; normal AS is zero to 64.

Patient No.	Day 0					Day 14				
	Heterophile	Guinea pig kidney absorption	Beef RBC absorption	ASO	AS	Heterophile	Guinea pig kidney absorption	Beef RBC absorption	ASO	AS
1	112	56	0	8	8	28	28	0	16	32
2	448	224	14	16	32	224	224	28	8	64
3	896	896	28	16	32	224	112	0	32	16
4	896	224	0	0	2	112	112	14	4	8
5	896			128	64	448	224	28	64	64
6	896	112	0	64	8	896	224	14	32	64
7	112	56	14	16	64	112	112	28	16	64
8	448	448	0	32	32	112	56	0	16	32
9	28,472	28,472	56	128	32	896	896	0	64	64
10	896	448	0	0	16	448	224	0	88	32
11	896	896	0	0	16	112	112	14	0	32
12	224	224	14	8	8	56	28	14	0	32
13	896	448	28	16	16	224	224	56	8	32

Fig. 2. Kidney biopsy from patient No. 9 on 9th day of acute illness. Specific fluorescence is focal within the glomerulus and appears to be in the cytoplasm of either the epithelial or endothelial cells, or both. Note diffuse cytoplasmic specific fluorescence of a granular nature in adjacent tubular cells. (× 270)

the positive heterophile fractions with beef red blood cells, thereby reducing the antibody titer against the latter to zero. Specific fluorescence was not affected by preabsorption of these fractions with guinea pig kidney tissue, which reduced the Forssman antibody titer significantly, but did not reduce the titer against beef red blood cells.

All other fluorescent control studies were completely negative.

Autologous heterophile-positive fractions always produced positive, specific fluorescent staining of the patient's own kidney tissue, and, in all cases but one, this would allow for specific fluorescent staining of the other patients' kidney tissues. The heterophile globulin obtained from patient No. 7 stained only autologous kidney tissue. It was also found that the heterophile fractions from the other 11 patients would not produce specific fluorescent staining of the kidney tissue of patient No. 7.

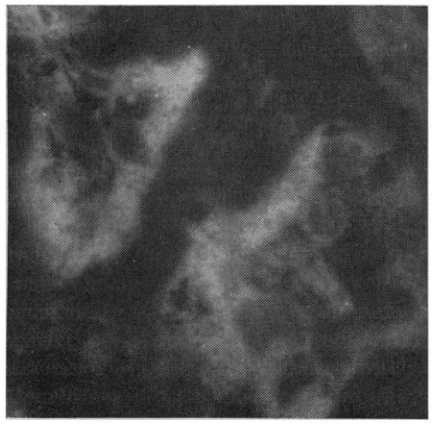

Fig. 3. Kidney biopsy from patient No. 13 on 10th day of acute illness. Specific fluorescence is localized throughout the cytoplasm of the tubular cells and has a granular appearance. (× 625)

The immunofluorescence results have several interpretations. If the antigen (or antigens) of beef red blood cells which allows for absorption of specific heterophile antibody is shared by the agent of infectious mononucleosis, then this cross reacting antigen of the infectious agent is probably what is being localized in the kidney tissue. Other possibilities are that the agent of infectious mononucleosis induces or uncovers a new antigenic determinant (or determinants) in the tissues which in turn induces the production of heterophile antibody. Such a new antigen could arise completely from the host tissue or could be a complex between infectious agent and host tissue. If the first view is correct, heterophile antibody could prove to be a useful tool in the isolation and identification of infectious agent in tissues and tissue cultures, and so forth. If the latter view were correct, heterophile antibody localization might be restricted if, for example, in a tissue culture system the new antigenic determinant(s) were different from that produced in vivo or did not appear at all. If infectious agent itself has been localized in the kidney tissue, it still remains to be determined whether its presence is due to active infection or whether it is merely being excreted into the urine. The general inflammatory changes found concomitantly would support active infection of the kidney, although some of these findings may be due to hypersensitivity.

It is unfortunate that other tissues, for example, tonsil, lymph nodes, and spleen, were unavailable for immunofluorescent staining in this investigation. Such a study might provide useful information supplementary to that presented in this report. Another possibly useful study would be examination of epithelial cells in the urine sediment by the immunofluorescent method. This might offer an easier means of identifying heterophile reactive antigen. This method could have potential value as a diagnostic and epidemiological tool.

The finding that the heterophile antibody from patient No. 7 had specificity for autologous kidney tissue only, whereas all the others showed complete cross reaction except with the agent of kidney tissue from patient No. 7, would speak for the possibility that two different strains of infectious mononucleosis exist.

The fact that beef red blood cells were capable of absorbing out heterophile antibody and removing specific fluorescent staining properties from all 12 heterophile-positive fractions would suggest that if two different strains of the agent of infectious mononucleosis did exist in this study, antigenic determinants from both strains were shared by the pool of beef red blood cells used.

A final note of interest is that the mild, diffuse inflammatory changes observed in the kidneys were detectable only by kidney biopsy and not by reasonably refined tests of renal physiology.

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