The details of these and related studies at present in progress will be described in further papers to be submitted for publication. The photograph (Fig. 1) was taken by Mr. E. Matthaei, to whom we wish to express our thanks.

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Rapid Extraction with Ethyl Acetate of Free Fluorescein Derivatives from Fluorescein *iso*Cyanate-Globulin Conjugates

The technique of staining tissues specifically with fluorescein-conjugated globulin has found wide application since it was introduced by Coons, Creech and Jones in 1942[1]. Though Coons and Kaplan[2] have recently introduced technical improvements the procedure remains in part tedious and time-consuming. Conjugation between fluorescein *iso*cyanate and protein is effected in a bicarbonate buffer (*p*H 9·0) in the presence of dioxan and acetone[2]; conjugation, however, is never quantitative and the presence of free dye in the protein solutions is inevitable. The free fluorescein derivatives must be eliminated since, when present in tissue sections being viewed with the fluorescence microscope, they impart a hazy appearance. At present the free fluorescein derivatives are removed from conjugates mainly by exhaustive dialysis and afterwards by adsorption with acetone-extracted or lyophilized mouse liver preparations. The dialysis is time-consuming, while in our experience prolonged adsorption with organ preparations removes specific conjugates as is shown by a fall in the antibody titre.

We have found that extraction of fluorescein-conjugated antisera with ethyl acetate at neutrality will remove from the reaction mixture both the free fluorescein derivatives and the acetone and dioxan. The following extraction procedure is used in this laboratory.

After the fluorescein *iso*cyanate and globulin have been allowed to react at *p*H 8–9 for 16 hr. at 0° C., the solution is extracted with 1 volume of ethyl acetate (A. R.) and the emulsion thus formed broken by low-speed centrifugation (1,000*g* for 5 min.). The supernatant acetate which contains the acetone and dioxan is discarded. The *p*H of the aqueous layer is then lowered to 7·0 (bromthymol blue) by the addition of *N* hydrochloric acid, and this solution extracted twice with 2 vol. of ethyl acetate. The supernatants are discarded and the dissolved acetate is removed from the aqueous phase *in vacuo* with a water pump.

During storage of either dialysed or acetate-extracted conjugated sera at − 10° C., there is a slow liberation of bound fluorescein derivatives which should be removed by a single acetate extraction before use.

Acetate extraction of serum globulin fractions did not affect the complement-fixing antibody titre of the rabbit and anti-rat-liver and anti-rat-kidney microsomal and mitochondrial sera which have been used in this laboratory.

While acetate extraction removes free fluorescein derivatives, it may be necessary, in addition, to adsorb the extracted serum with the appropriate heterologous antigen—often a dried acetone-extracted organ preparation[2] or a fresh particulate tissue fraction[3]—in order to yield a serum with the required specificity.

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Clearing Action of Lysolecithin

G. R. Webster has recently reported very interesting experiments on the clearing action of lysolecithin on brain homogenates[1]. Analogous findings with lysolecithin (and animal venoms) on brain brei suspended in physiological saline were published in 1942 by Grosse and Tauböck[2].

The solubilizing effect of lysolecithin may be observed also with suspensions of cholesterol, lecithin, oleic acid and particle preparations from liver[3] and on diluted egg yolk[4]. It may be connected with the high surface activity of lysolecithin[3].

Lysolecithin acts on enzyme systems, for example, of oxidative phosphorylation[5], succinoxidase system[6] and on tissue thromboplastin[6], possibly by disintegrating biological structures. Complete lysis of erythrocyte stroma[7] and changes of morphology of liver mitochondria[8] have been shown.

Lysolecithin has been sometimes thought to be involved not only in the normal or pathological turnover of blood cells (for review, see ref. 9) but also in pathological conditions of the central nervous system[10]. If lysolecithin, or the phospholipase *A* producing it, is present in the body, it may be very important for permeability and solubilizing processes of all kinds[3]. Knowledge of the presence of lysolecithin or phospholipase *A* in normal or pathological tissue, however, is very restricted.

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