There is no case with tumors in the thoracic cavity. Tumors present on the abdominal side of the diaphragm may infiltrate between muscle fibres, but the thoracic side of the diaphragm remains always smooth and free of fibrous nodules. No tumors are found at the site of injection and only very exceptionally at the site of subcutaneous implantation of a tablet of estradiol.

11838 P

Studies on Fluorescence Associated with Proteins.

WENDELL REEDER AND V. E. NELSON.

From the Laboratories of Physiological Chemistry, Iowa State College.

The relative fluorescence intensities of the proteins and their hydrolysates were determined by measuring the dilution required to reduce the fluorescence of a given amount of protein or protein hydrolysate to the same intensity as the fluorescence of a diluted standard solution of quinine bisulfate.

The proteins prepared and studied in this investigation were: casein, wheat gluten, gliadin, glutenin, blood fibrin, gelatin, ovalbumin and zein. Hair and wool were also compared to the above proteins.

When examined in ultraviolet light of wavelengths 3100-4100 Å the proteins give a uniform bluish-white fluorescence in the solid state and a somewhat more green fluorescence in solutions. The fluorescence of these proteins is more green in basic solution than in acid, but the color change is not sharp.

Fluorescence of proteins is destroyed by oxidation with strong nitric acid or by ashing. The small amount of protein ash is not fluorescent in the solid state nor in acid, basic or neutral solution.

Organic solvents do not extract the fluorescent material from the solid protein nor from the protein hydrolysates in acid or basic solution. Likewise, dialysis experiments failed to remove the fluorescent material from protein solutions but after hydrolysis with strong acid the fluorescent material is readily removed from proteins by dialysis.

Hydrolysis of proteins by proteolytic enzymes or alkali produced only a slight increase in the amount of fluorescence. However, hydrolysis with hydrochloric acid, sulfuric acid, perchloric acid or phosphoric acid produced large increases in the fluorescence of those

proteins containing tryptophane and only slightly increased fluorescence in those proteins which are deficient in this amino acid. The color of the fluorescence produced during acid hydrolysis is blue-green.

Crude commercial proteins produce the same amount of fluorescence, during acid hydrolysis, as these same proteins prepared in a purified state. The presence or absence of air during acid hydrolysis does not affect the amount or color of fluorescence of the protein hydrolysates.

Nineteen amino acids were examined in ultraviolet light for fluorescence. These amino acids were not noticeably fluorescent in the solid state nor in acid, basic or neutral solution.

Amino acid additions to proteins during hydrolysis revealed that tryptophane was the only amino acid which increased the amount of fluorescence during acid hydrolysis. Tryptophane did not affect the amount of fluorescence produced during hydrolysis of proteins with alkali.

When proteins such as zein and gelatin, which are deficient in tryptophane, are hydrolyzed with acid in the presence of this amino acid the amount of fluorescence is greatly increased. However, when tryptophane is added to proteins containing this amino acid the fluorescence is only slightly increased during acid hydrolysis.

The addition of the vitamins which are capable of producing blue-fluorescent compounds did not affect the amount or color of fluorescence during acid hydrolysis.

Boiling acetic acid with proteins greatly increased the amount of fluorescence. However, the fluorescent color produced is much more blue than that of protein solutions or hydrolysates. Boiling acetic acid with several of the amino acids caused a blue fluorescence to appear in the solutions. Strong acids do not produce fluorescence of amino acids. This would indicate that the fluorescence increase with acetic acid and protein is due to a different substance than is produced during acid hydrolysis with strong acids.

Tyrosine and tryptophane were the only amino acids of all those studied which gave blue-green fluorescence with glucose when boiled with dilute HCl. The melanin produced by the action of the tyrosinase of potato juice on pure tyrosine solutions was not fluorescent.

Concentration of the fluorescent material was accomplished by adsorption of the material from acid solutions with English fuller's earth (Cenco fuller's earth was a poor adsorbent) and subsequent elution from this adsorbent with ethyl or methyl alcohol-ammonium hydroxide solutions. From its solubility properties the fluorescent

material was found to be different from the blue-fluorescent alkaloid harman produced by the mild oxidation of the acetaldehyde-tryptophane complex. Harman when injected intraperitoneally into rats caused paralysis of the hind legs of the animals and slowing of heart action whereas injection of neutralized protein hydrolysate or the adsorbed material from the hydrolysate had no effect.

Lactoflavin, thiochrome, quinine bisulfate and the fluorescent material from protein hydrolysates give broad bands of fluorescent light. The fluorescent spectrum of the material concentrated from casein hydrolysate is in the violet-green region of the spectrum and is very similar to the fluorescent spectrum of quinine bisulfate. The fluorescent spectra of lactoflavin and thiochrome are different from the fluorescent spectrum of protein hydrolysate. The fluorescent spectrum of the material concentrated from casein hydrolysate is in the violet-green region having wavelengths 4100-5300 Å. The fluorescence of protein hydrolysates is excited only by light of wavelengths 3400-3600 Å.

11839 P

Production of Pernicious Anemia-like Syndrome in Rats with Bile Acids.

RAPHAEL ISAACS.

From the Hematology Laboratory, Michael Reese Hospital, Chicago.

Because of the property of bile acids to produce hemolysis of red blood cells, cytolysis of leukocytes, destruction of nerve cells and myelin of nerve fibers, as well as to produce gastric lesions, a group of symptoms present in pernicious anemia, 12 rats were injected subcutaneously from one to 6 times a week with a suspension of glycocholic acid from ox bile The effective daily dose after trials with weaker suspensions, was 10 mg. Of 12 rats, 3 developed a mild macrocytic, oval red blood cell anemia during the course of 2 months. During this time, one developed dragging of the hind legs, with a clumsy gait, and in 3 months, 2 showed skin ulcerations in regions which had not been used for injections. These symptoms did not appear in a control group, nor in a group injected with liver extract at the same time as the glycocholic acid.

Table I shows the nature of the blood changes.

The enlargement of the red blood cells appeared before any marked