possible, however, to identify these transplanted tumors as nephromas. Only occasionally are there areas suggestive of abortive tubule formation, and no glomerulus-like formations have been observed.

Intraperitoneal transplantation into the 5- to 11-day-old rats produced lethal tumor growth in all of the animals injected with a million or more cells. Smaller numbers of cells were not tested i.p. There was no evidence of growth on i.p. transplantation into 18- to 34-day-old rats but only 2 rats were tested with more than 1 million cells.

The intraperitoneal tumors grew as nodules and sheets of tumor cells attached to the peritoneal surfaces of diaphragm, omentum, and abdominal viscera. Histological examination revealed some areas of direct invasion through the peritoneum into underlying viscera (Fig. 3) but usually growth was not infiltrative.

Summary. An embryonal nephroma, which occurred spontaneously in a rat and had been maintained by serial transplantation, has now been established in tissue culture as a stable cell line and has been maintained in continuous passage for more than 5 years. It is readily transplantable to young rats, subcutaneously or intraperitoneally, by inoculation of a million or more cells but such transplants often regress spontaneously.

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The "Free" and "Buried' Tryptophan and Tyrosine Residues of Human Plasma α_1-Acid Glycoprotein.* (31706)

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The determination of the "free" and "buried" amino acid residues of proteins is known to contribute greatly to our understanding of the structure of this class of biologically important macromolecules. Recently, such studies have been reported for certain enzymes(1,2,3,4) relating activity to some of their "free" amino acid residues. However, apparently no glycoprotein has been investigated by such techniques. In this report we present the results of the determination of the "free" tryptophan and tyrosine residues in α_1-acid glycoprotein (orosomucoid)(5,6), a well characterized glycoprotein of normal human plasma.

2-Hydroxy-5-nitrobenzylbromide which has been demonstrated by Koshland *et al*(3) to be a highly specific reagent for "free" tryptophan residues, and native α_1-acid glycoprotein isolated from Cohn Fraction VI of pooled normal human plasma(5) were used for the first part of this study. The sialic acid-free glycoprotein was prepared by treatment of the native protein with neuraminidase. The reaction of α_1-acid glycoprotein with this reagent was carried out essentially as described by Koshland(3,8). In certain experiments the solvent contained increasing amounts of 2-chloroethanol. The excess of the reagent and solvent was removed by filtration through a Sephadex G-25 column. The effluent containing the protein was dialyzed against water and lyophilized. An aliquot of the resulting protein powder was dissolved in 0.1 N NaOH and its absorbency was determined at 410 mμ. Using the molar extinction coefficient of 18,900 for the Koshland reagent(3), the

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TABLE I. "Free" Tryptophan Residues of Native α_1-Acid Glycoprotein in Different Solvents.

2-Chloroethanol, %	No. of residues per mole of protein	Specific optical rotation $[\alpha]^{23}_{516}$
0	1.9	—26°
20	2.5	—43°
50	3.7	—24°

number of "free" tryptophyl residues was calculated from these measurements.

The results obtained are summarized in Table I. In aqueous solution 2 of the 4 tryptophyls of native α_1-acid glycoprotein were found to be "free." In the presence of 50% chloroethanol, the remaining 2 tryptophan residues also reacted with this reagent. Essentially identical results were obtained when the sialic acid-free form of this glycoprotein was investigated. This study further suggests that the carbohydrate moiety does not interfere with these determinations on glycoproteins. Optical rotation measurements of α_1-acid glycoprotein also demonstrated the changes in the structure of this protein in presence of chloroethanol.

In the second part of this study, the "free" tyrosine residues of α_1-acid glycoprotein were determined. The method of Vallee and coworkers(9) involving the reaction with acetylimidazole at pH 7.5 was used. In aqueous solution 5 of the 12 residues(10) reacted with this reagent. In presence of 8 M urea without change in pH essentially all residues were found to be "free." The optical rotation of α_1-acid glycoprotein in 8 M urea was reported earlier to be increased to 64° in a reversible fashion(11).

Summary. The "free" tryptophan and tyrosine residues of α_1-acid glycoprotein (orosomucoid) were determined with the highly specific reagents of 2-hydroxy-5-nitrobenzylbromide and acetylimidazole, respectively. In the native protein 2 of the 4 tryptophyls and 5 of the 12 tyrosyls were "free." In the presence of 50% 2-chloroethanol or 8 M urea, essentially all these residues reacted with the reagents mentioned.

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Inhibitory Effect of Chlorothiazide *in vitro* on Glucose Metabolism of Adipose Tissue.* (31707)

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Chlorothiazide added directly to the incubating medium reduces the *in vitro* rate of utilization of glucose by epididymal fat pads of rats(1). This inhibitory action of chlorothiazide was demonstrated with high concentrations of the drug. The effect *in vitro* of lower chlorothiazide concentrations, therefore, is presented here.

Methods. Six experiments were carried out in which the rate of utilization of glucose

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