ganic acids (*6*), where a proton transfer from a counterion or a reagent mixed with the salt leads to the formation of a neutral compound which can then be vaporized. However, although the exact mechanistic details need further study, this technique can be directly applied to specific analytical problems.

Our results demonstrate the compatibility of a model ion pairing system with MS analysis and its compatibility with on-line LCMS. This technique provides a convenient method for both the separation and MS analysis of ionic compounds. Moreover, the data demonstrate the ability to convert ionic compounds into a volatile form by ion pair derivatization. Significantly, the resulting spectra correspond to those of the unpaired material and contain no mass increment as is usually the case with ordinary derivatization schemes. The ion pairing technique should be readily adaptable to the analysis of many sulfate and sulfonate surfactants and eventually should be extendable to other classes of ionic compounds.

Since the completion of these experiments, we have evaluated the on-line LCMS analysis of ion pairs. A system consisting of a continuous extraction interface and moving belt inlet to the MS, as described in (*2*), was used. The resulting spectra (both EI and CI) were identical to those shown here, and no detrimental effects on the operation of the MS were observed (*7*).

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Human Hepatocellular Carcinoma Cell Lines Secrete the Major Plasma Proteins and Hepatitis B Surface Antigen

Abstract. *Analysis of the cell culture fluid from two new human hepatoma-derived cell lines reveals that 17 of the major human plasma proteins are synthesized and secreted by these cells. One of these cell lines, Hep 3B, also produces the two major polypeptides of the hepatitis B virus surface antigen. When Hep 3B is injected into athymic mice, metastatic hepatocellular carcinomas appear. These cell lines provide experimental models for investigation of plasma protein biosynthesis and the relation of the hepatitis B virus genome to tumorigenicity.*

Plasma protein synthesis, a specialized function of liver parenchymal cells, has been studied in vitro in liver slices from experimental animals (*1*), in suspension and in primary cultures of isolated hepatocytes (*2–6*), and in hepatoma-derived cell lines (*7–9*). From these investigations, general principles for the control of some of these liver-specific functions in rodents have been derived. However, no experimental system for the investigation of plasma protein synthesis in the human has been described.

We have isolated two human cell lines (Hep G2 and Hep 3B) from liver biopsies of two children with primary hepatoblastoma and hepatocellular carcinoma (*10*). We found that 17 of the major plasma proteins are secreted into cell culture medium. A previously described (*11*) hepatoma cell line (PLC/PRF/5) produces seven of the same human plasma proteins. The Hep 3B and PLC/PRF/5 cell lines synthesize hepatitis B virus surface antigen (HBsAg) (*10, 11*); the Hep G2 line does not synthesize HBsAg. We found that the two major polypeptides of HBsAg are synthesized by Hep 3B and PLC/PRF/5. Hepatitis B virus (HBV) has been epidemiologically implicated as a probable causative agent of the majority of hepatocellular carcinomas (*12*). We now report that Hep 3B and PLC/PRF/5 form tumors when injected into nude mice. These cell lines thus provide an excellent model for the investigation of human plasma protein synthesis and the hepatitis B virus–host cell relationship.

Table 1. Identification of plasma proteins in tissue culture medium of human hepatoma cell lines by the Ouchterlony double-diffusion technique.

Human protein	Reaction with antiserum*		
	Hep G2	Hep 3B	PLC/ PRF/5
α-Fetoprotein	+	+	−
Albumin	+	+	−
α_2-Macroglobulin	+	+	+
α_1-Antitrypsin	+	+	+
α_1-Antichymotrypsin	+	+	−
Transferrin	+	+	+
Haptoglobin	+	+	−
Ceruloplasmin	+	+	+
Plasminogen	+	+	+
Gc-globulin	−	+	−
Complement C3	+	+	+
Complement C4	+	−	−
C'3 activator	+	+	−
α_1-Acid glycoprotein	+	+	+
Fibrinogen	+	+	−
α_2-HS-glycoprotein	+	+	−
β-Lipoprotein	+	+	−
Retinol-binding protein	+	+	−

*Indicates line of identity with either normal human plasma or human placental cord serum. Negative controls include culture fluid from human fibroblasts and mouse cells. Samples taken from confluent flasks, which contained the same culture fluid throughout the growth cycle of the culture (7 to 14 days), were concentrated tenfold by use of Minicon-B15 concentrators (Amicon, Lexington, Mass.). No reactivity was noted with antibody to prealbumin and hemopexin. All antibodies were purchased from Cappel Laboratories (Cochranville, Pa.) or Calbiochem-Behring (San Diego, Calif.). The amounts of albumin and α-fetoprotein produced by cultures initiated with 10^6 Hep 3B cells and grown for 2 weeks without medium change (1.1×10^7, final cell number) are 300 and 500 μg, respectively (*10*).

The Hep G2 and Hep 3B cell lines were initially derived from tissue minces of hepatoma biopsies placed on feeder layers of the irradiated mouse cell line STO (*13*) in William's E medium (Gibco) supplemented with 10 percent fetal bovine serum. This method of isolation promotes the growth of cells that have fastidious requirements while preventing fibroblastic overgrowth. After an initial period of apparent cell proliferation, growth was restricted to single colonies that were maintained in flasks for several months. Cells from flasks containing single large colonies were dissociated by trypsinization (0.25 percent trypsin, 0.1 percent EDTA in phosphate-buffered saline) and transferred to new feeder layers, eventually producing proliferating cell lines that have now been serially passaged more than 50 times. Sublines of both Hep G2 and Hep 3B have been selected for feeder independence. These cell lines resemble liver parenchymal cells morphologically and are chromosomally abnormal: Hep G2, $\bar{X} = 55$ (50 to 56); Hep 3B, $\bar{X} = 60$, with a subtetraploid population, $\bar{X} = 82$. Both cell lines contain distinctive rearrangements of chromosome 1.

When Hep 3B was injected under the

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kidney capsule of athymic (nude) mice, tumors appeared that were histologically similar to the hepatoma from which the cell line was derived. Numerous metastases were noted in the lungs of one mouse injected with Hep 3B; at autopsy, HBsAg (approximately 0.1 mg/ml) was found in the serum of this tumor-bearing mouse. PLC/PRF/5 also formed tumors in nude mice when injected under the kidney capsule. No tumors have been observed when Hep G2 cells were injected.

Supernatant fluid from confluent cultures of both Hep G2 and Hep 3B was analyzed by the Ouchterlony immunodiffusion technique (*14*) with commercially available antiserums to the major human plasma proteins (Table 1). Of the 20 plasma proteins surveyed, Hep G2 produced all but Gc-globulin, prealbumin and hemopexin, and Hep 3B produced all but the fourth component of complement, prealbumin, and hemopexin. Only seven of these plasma components were produced by PLC/PRF/5. Albumin and α-fetoprotein (AFP), which are major secreted products of the Hep 3B and Hep G2 cell lines (*10*), are not detectable in PLC/PRF/5 supernatants [see also (*15*)]. For further identification of some of the plasma proteins, confluent cultures were labeled with [^{35}S]methionine, and the supernatant fluid was analyzed by the two-dimensional gel electrophoretic technique of O'Farrell (*16*). Comparison of the isoelectric points and molecular weights of the proteins seen on autoradiograms of the two-dimensional electrophoretic gels with those of Coomassie brilliant blue-stained normal human plasma (*17*) allows positive identification of some of the secreted proteins detected by immunologic techniques (Fig. 1). Autoradiograms of gels from PLC/PRF/5 cell culture fluid show a few secreted plasma proteins (Fig. 1a), whereas Hep G2 and Hep 3B cells secrete many proteins (Fig. 1, b and c) identical to those found in human plasma (Fig. 1d). The PLC/PRF/5 cell line when assayed by the Ouchterlony method does not appear to be making albumin or AFP, but trace amounts of label are found in the albumin-AFP region of the PLC/PRF/5 gel (Fig. 1a). We have previously reported differences in the amounts of AFP and albumin secretion throughout the growth cycle of the Hep 3B cell line (*10*). It is possible that trace amounts of these proteins may be made by PLC/PRF/5 during a specific portion of the growth cycle. The same mechanism may explain the absence (during a 5-hour pulse) of labeled proteins that are shown to be present by Ouchterlony analysis of supernatant fluids from long-term cultures.

The amount of HBsAg in the culture supernatants of PLC/PRF/5 and Hep 3B is insufficient for the direct identification of ^{35}S-labeled HBsAg on the two-dimensional gels. However, when larger amounts of ^{35}S-labeled cell culture fluid are reacted with antibody to HBsAg and

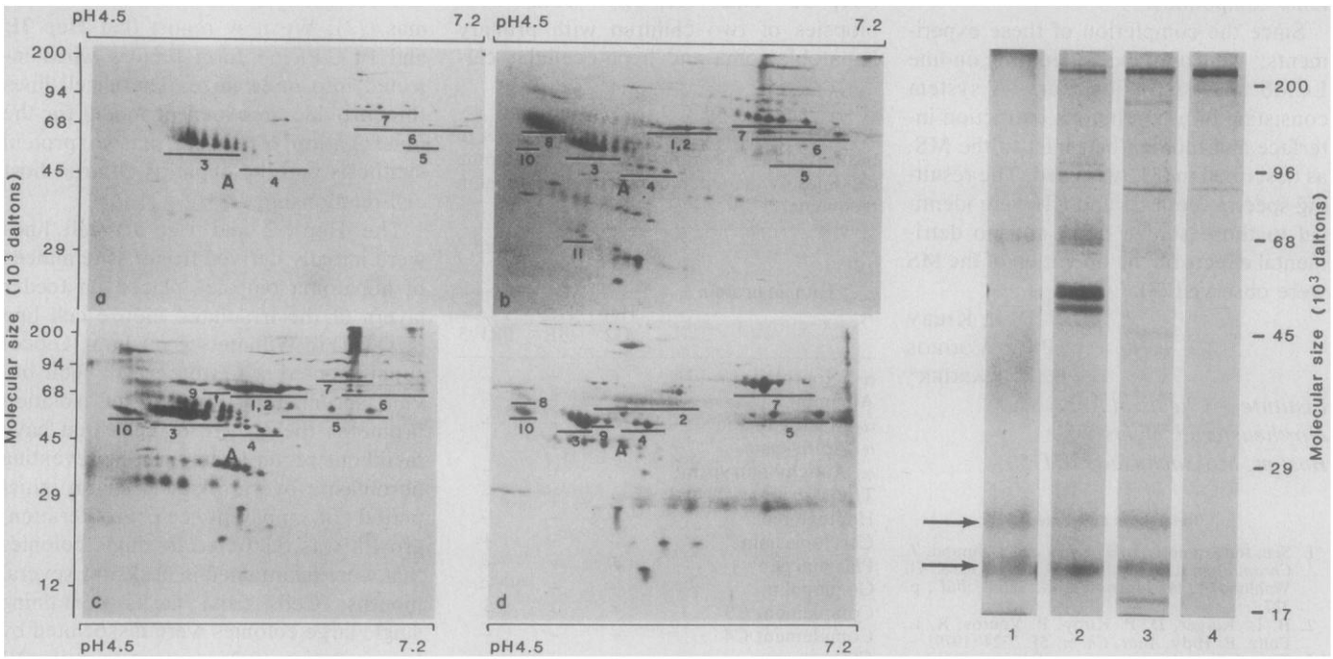

Fig. 1 (left). Two-dimensional gel electrophoretic patterns. (a) Autoradiogram of ^{35}S-labeled culture fluid from PLC/PRF/5 cells (53,000 count/min applied in 10-μl sample; 10-day exposure). (b) Autoradiogram of ^{35}S-labeled culture fluid from Hep G2 (148,000 count/min in 10-μl sample; 5-day exposure). (c) Autoradiogram of ^{35}S-labeled culture fluid from Hep 3B (98,000 count/min in 10-μl sample; 7-day exposure). (d) Coomassie brilliant blue-stained human plasma (2-μl sample). Supernatants were collected after 5-hour incubation of confluent cell cultures in T75 flasks (Falcon Plastics) with 1 mCi of [^{35}S]methionine (NEG 009T) in 5 ml of Eagle's minimal essential medium supplemented with 5 m*M* glutamine. Albumin was removed from fresh heparinized plasma by passage through an Affigel Blue (Bio-Rad) column prior to sample preparation. Samples of ^{35}S-labeled cell culture supernatant fluid and albumin-deficient plasma were mixed with 50 μl of lysis buffer (*16*) and separated by two-dimensional electrophoresis (*16*). Positions 1 and 2, albumin and α-fetoprotein; position 3, α_1-antitrypsin; position 4, fibrinogen (γ chain); position 5, fibrinogen (β chain); position 6, fibrinogen (α chain); position 7, transferrin; position 8, α_1-antichymotrypsin; position 9, Gc-globulin; position 10, α_2-HS-glycoprotein; position 11, complement C4; *A*, actin (the tip of the *A* points to the actin spot). Fig. 2 (right). Autoradiogram of immunoprecipitates from cell cultures labeled with [^{35}S]methionine (see legend to Fig. 1). Linear gradient electrophoresis was performed on a 7.5 to 15 percent polyacrylamide-sodium dodecyl sulfate gel slab (*21*). Purified HBsAg (*22*) was iodinated by the chloramine-T method (*23*). ^{35}S-Labeled cell supernatant (1 ml) or ^{125}I-labeled HBsAg (0.5 μg in 0.8 ml of phosphate buffer) was incubated (5 hours at 4°C) with 10 μl of guinea pig antiserum to HBsAg/ad (*24*); 20 μl of rabbit antiserum to guinea pig immunoglobulin G (Miles Laboratories) was added, and the mixture was incubated (3 hours at 4°C). Immune complexes were absorbed onto *Staphylococcus aureus* Cowan I, washed according to the method of Cullen and Schwartz (*25*), and eluted, reduced, and prepared for electrophoresis (*26*). Lane 1, ^{125}I-labeled HBsAg (10,000 count/min); lane 2, [^{35}S]methionine-labeled PLC/PRF/5 (24,000 count/min); lane 3, [^{35}S]methionine-labeled Hep 3B (20,000 count/min); and lane 4, [^{35}S]methionine-labeled Hep G2 (15,000 count/min).

the immunoprecipitates are analyzed on sodium dodecyl sulfate-polyacrylamide gels, two bands are observed (Fig. 2, lanes 2 and 3) that, on electrophoresis, migrate with the P-1 and P-2 polypeptides immunoprecipitated from ^{125}I-labeled purified HBsAg (Fig. 2, lane 1). These bands are two of the seven polypeptide components reported for HBsAg (*18*) and correspond to the p23 and p28 components identified from purified 22-nm particles and the PLC/PRF/5 cell line (*15*). The additional bands observed in lanes 2 and 3 are nonspecific and are observed in the absence of antiserum to HBsAg. The one exception to this is the 68,000-dalton band that is thought to be copurified albumin (*19*). The P-1 and P-2 subunits are probably the only major viral components of HBsAg (*15, 20*). No HBsAg components were found in the cell culture medium from Hep G2 (Fig. 2, lane 4).

One of the most interesting aspects of these tumor-derived cell lines is that they have the biosynthetic capabilities of normal liver parenchymal cells. Indeed, the Hep 3B and PLC/PRF/5 cell lines are capable of tumor formation in athymic mice. We currently have no evidence for the presence of the hepatitis B viral genome in the Hep G2 cells, even though the line is phenotypically similar to the Hep 3B cell line. The retention of the differentiated liver cell functions in this replicating cell line does not therefore relate to the presence of the viral genome. Quantitative comparison of the production of plasma proteins and HBsAg by Hep 3B cultures indicates that the expression of the viral coded product is regulated by the host cell (*10*). The availability of these human parenchymal cells in continuous culture, synthesizing a large number of readily identifiable secreted products, should provide a major tool for investigation of the control of their biosynthesis.

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Cerebral Regional Oxygen Consumption and Supply in Anesthetized Cat

Abstract. *The study involved quantitative measurement of arterial and venous oxygen saturation, oxygen extraction, blood flow, and oxygen consumption in specific areas of the brain. No regional differences in oxygen consumption were found in anesthetized cat brain, and the amount of oxygen available to all regions studied was more than 2.5 times the consumption throughout the brain.*

Roy and Sherrington (*1*) were the first to propose that brain blood flow was regulated to meet the requirements of metabolism. This hypothesis was never tested on a quantitative basis in different brain regions because of limitations of existing technology. Our investigation represents, to our knowledge, the first quantitative determination of the normal regional relationship between oxygen supply and consumption in the brain.

Some areas of the brain are known to be susceptible to oxygen lack, even for a short time. It would be interesting to determine which areas are more susceptible than others. Estimates that may be used as indicators of regional metabolic rates are available for regional oxygenation (*2*), reduced nicotinamide adenine dinucleotide, and cytochrome aa_3 oxidation state (*3*) in various parts of the brain. These methods provide only an estimate of oxygen supply with respect to consumption rate rather than a measure of oxygen supply and consumption.

We report a new microspectrophotometric method for determining oxygen saturation in small blood vessels of quick-frozen brain. Combining this measure with regional blood flow measured by the radioactive microsphere method, we have determined oxygen consumption in defined regions of the brain by application of the Fick principle. No other methods are available for determining oxygen consumption in such small areas and deep structures of the brain.

Unlike most other organs, the brain is a conglomeration of a large number of diverse functional areas. At any moment some of these units may be active and others quiescent. The energy need and, therefore, the metabolic rate of a given region are related, in part, to the instantaneous amount of activity. Some in vivo measurements of regional metabolic rate in the brain may be used as an index of average neuronal activity in that region. The recognized usefulness of monitoring the metabolic rate in the brain in relation to mental activity has led to the development of several procedures; however, attempts to measure oxygen consumption, glucose utilization, or oxygenation are limited to localized brain regions, are indirect, or are not quantitative measures of regional brain oxygen consumption (*2-5*).

Our method is a technique for determining oxygen consumption in small areas of superficial and deep structures of the brain. The arterial-venous oxygen extraction can be determined by a microspectrophotometric method by examining small vessels regionally in quick-frozen tissue (*6, 7*). This method is highly accurate and—since we looked at blood within blood vessels—independent of the organ studied. We measured blood flow through the use of radioactive mi-

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