Ray Peat's Newsletter

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THE ARGUMENT:

Cancer is the result of ordinary physiological processes which become autonomous because of regulatory weaknesses in the organism.

Respiration is essential for the maintenance of the higher forms of life, and it is a respiratory defect, on both the cellular and the organismic levels, which allows cancer to persist and develop.

The heme group, because it serves many respiratory functions--hemoglobin, mitochondrial respiratory enzymes, steroid synthesizing enzymes, formation of thyroid hormone, detoxifying enzymes--is regulated in relatively primitive ways within each cell, and in more complex ways at higher organismic levels.

When the cell needs more respiratory energy, some fuel is diverted into the production of porphyrin, which is then turned into heme, which would normally provide for the efficient production of energy and protective factors.

When the efficient energy-producing systems are blocked, by injury, oxygen deficiency, toxins, or by the lack of one or more essential nutritional factors, heme production is activated.

Excess heme is destroyed by the enzyme heme oxygenase, which converts heme into biliverdin and carbon monoxide. Both of these factors have effects on the cell which are characteristic of cancer.

Estrogen, radiation, chemical carcinogens, and other forms of stress, activate the heme oxygenase enzyme.

Estrogen causes both porphyria and jaundice and is associated with increased formation of carbon monoxide. It inhibits many types of liver function, including detoxification.

The production of carbon monoxide by cancer cells can account for cancer's self-sustaining, "hereditary," property, without invoking genetic mutations which are now known to be consequences, rather than causes of cancer.

The production of carbon monoxide and biliverdin can account for many of the structural and biochemical abnormalities of cancer cells, and for their induction of abnormalities in adjacent cells.

"Genetic" theories of cancer have now reached a dead end, and the epigenetic, developmental-physiological approach remains as the only plausible description of cancer.

Carbon Monoxide, Stress, and Cancer: 1999 status

When we thoroughly understand cancer, we will be closer to understanding life itself.

Cancer, AIDS, and extreme hypothyroidism have several features in common--they cause tissue loss and organ damage, with immunodeficiency and intense activation of the stress hormones, including cortisol. In cancer and AIDS, a good case has been made for the primacy of stress-induced wasting as the main cause of death. Whatever one might believe to be the cause of cancer and AIDS, it is always good for the patient to prevent tissue damage from the stress associated with the sickness. Since the stress hormones primarily destroy tissues by the activation of specific proteases, the use of protease inhibitors for treating AIDS is probably helping to prevent tissue loss, as an unintended side effect. But in the case of cancer, there are good reasons for believing that metabolic dysregulations are the cause of the disease itself, as well as of the wasting symptoms.

When I wrote about carbon monoxide as a cancer hormone a few years ago, some people were confused, and I think that was because I didn't explain in detail how Otto Warburg's work made the idea seem so obvious.

In studying the pigments of cellular respiration (enzyme cofactors, including vitamin B2 and the cytochromes), Otto Warburg (1926) found that carbon monoxide, the respiratory poison, bound to some of the crucial pigments, and that light would free the pigment from the carbon monoxide, restoring respiration. Despite the fact that his work is described in all biochemistry textbooks, until very recently the toxicity of carbon monoxide was ascribed by medical texts only to its

effects on hemoglobin, preventing the delivery of oxygen to cells, rather than to its effects on the respiratory pigments in the mitochondria, as demonstrated by Warburg. Of the thousands of publications on the toxicity of carbon monoxide in recent decades, its effects on mitochondrial metabolism have hardly been investigated, because of the persepective that it was a poison coming from outside the body, and that its effect on hemoglobin was sufficient to cause death, so the question of its effect on the mitochondrion was medically irrelevant.

In his famous theory of cancer, Warburg argued that cancer was always characterized by "a respiratory defect," and he argued that many things could damage the respiratory system and lead to cancer. He and his colleagues over several decades showed that all the known causes of cancer produced the characteristic respiratory defect of cancer, in which cells would produce lactic acid even in the presence of normal levels of oxygen. This is called *aerobic glycolysis*, and in normal cells, the production of lactic acid is *anaerobic*, since the availability of oxygen suppresses the production of lactic acid, and causes carbon dioxide to be produced instead. (This effect of oxygen is called the Pasteur effect, and I think it is largely the production of carbon dioxide which accounts for it, though other things such as the level of ATP contribute to it.)

Warburg showed that chemical carcinogens, radiation, prolonged culture without adequate oxygen, and even vitamin B1 or B2 deficiency would promote first the characteristic cancer metabolism, and then actual cancerization. Most cells with the respiratory defect simply died, and it was those which could adapt to live with the defect which became cancerous. The tumor which results from a metabolic defect will "epigenetically," secondarily, develop chromosome imbalances, which may approximately coincide with the cells' transformation into cancer, and the conditions within the tumor will increase the tendency to produce mutations, which are not likely to be repaired in the extremely stressed state of the cells.

I think the reason Warburg didn't explore the role of carbon monoxide in cancer was simply that, during his lifetime, it wasn't recognized as something which is produced in the body. **Warburg had no reason to suspect that carbon monoxide would be produced by the cell exactly when the cell's respiratory apparatus was challenged. But since about 1971, carbon monoxide has been recognized as a normal product of the degradation of heme, approximately at the same time that it has become clear that respiratory distress on the cellular level stimulates both the synthesis and the degradation of heme.**

Unfortunately, after Warburg's death, there were no prestigious advocates of his theory, and his death coincided with America's "war on cancer," which consisted of giving huge public funding to the "cancer genetics" estrablishment, people who were congenitally hostile to the concept that cancer had knowable or controllable causes. Industry and medicine found it convenient to argue that people were responsible for their own diseases, in the sense that they inherited them, as a kind of mechanistic karma. *Mutations in the inherited genes were, by definition, "random," which gave them the virtue of being impossible to pin down to a specific, legally actionable, cause.*

The genetic, and thus "essential," nature of cancer justified the concept of treating it by killing it. (The procedure William Blake described 200 years ago as "curing the pox by cutting out the spots.") The doctrine of genetic causality indicated that only capital punishment could deal with the traitor-genes. Although these essentialists at first had rejected the idea that viruses could integrate themselves within our genes, after several years they realized that this idea meant that the infectious disease establishment could join forces with the genetics establishment. The new, highly funded "oncogene" establishment had absolute control over cancer research, and that direct control spread indirectly, like a malignancy, throughout academic and commercial biology.

Most people in the contemporary medical industry are so fond of the idea of DNA-mutation, followed by "clonal selection," to explain cancer, that they are able to ignore the many types of

evidence that show cancer to be a process of deterioration in a mass of cells, rather than a progression of a single cell through stages of DNA mutation.

This obsessive belief that "DNA causes everything" goes back to Weismannism, and the Neo-Darwinism that he founded by presenting his--purely arbitrary--arguments to eliminate the inheritance of acquired traits from the theory of evolution, and--*opposing Darwin*--to claim that Natural Selection was the only force in evolution. Weismannism, in its absolute rejection of the environmental adaptationism of Darwin and Lamarck, was almost a reversion to Preformationsim. In its insistence on the "isolation of the germline," or the *immortality of the germline* which was passed on in the germ cells of the gonads, Weismannism subscribed to the Preformationist belief that all future generations are contained in the germ cell, and its adherents tried to justify this by asserting that the *soma,* all the rest of the individual outside of the germ cells, was nothing more than a mortal shadow of the essential (genetic) immortal being, since the body was produced precisely by the **deletion** of the information contained in the germ cell. (This doctrine was behind the denial that animals could be cloned from the nucleus of body cells. Even after frogs, sheep, and cows were produced in this way, there were some people who couldn't accept the results as facts. The doctrine that "telomeric shortening" causes aging came straight out of Weismannism.)

Of course those ideas were false, nothing but an absurd application of Rationalist philosophy to biology to eliminate Darwin's "materialism," but the doctrine's importance is undeniable, since its direct influence can be seen in every university biology course and textbook of the 20th century.

It is this essentialist commitment of the cancer establishment that has made Warburg's work practically disappear from the academic culture. His demonstration that the cancer metabolism could be reversed by vitamins was a threat to the entire foundation of twentieth century essentialist medicine.

Poor Nutrition + Estro
= ↑ porphyrin

Because of my interest in the effects of light on the hormones and respiratory energy production, and my long-standing interest in Warburg's view of oxygen's role in evolution and cancer, and my awareness that *porphyrins** were at one time thought to be cancer hormones, it seemed obvious to think of carbon monoxide as a cancer hormone.

I happened to become acquainted with Leonell Strong, who developed the strains of cancer-prone mice that have been used in breast cancer research. ***Although he was a geneticist and bred the mice that demonstrated the inheritance of cancer, he also showed that simple therapeutic measures would not only prevent cancer in a susceptible individual, but would also prevent cancer in many generations of the treated animal's descendants.*** Strong discovered that estrogen and poor nutrition lead to overproduction of the porphyrin pigment, and contribute to the development of cancer. He showed that the liver was involved in the control of cancer. The Shutes, the Biskinds, and Alexander Lipshutz were at this time revealing other effects of estrogen that illuminated Strong's discoveries.

Besides Strong, who showed individual and hereditary avoidance of cancer, many other workers have demonstrated that "established" cancer cell lines can be induced to revert to normal tissue. Inheritance isn't indelibly fixed. A *physiological state* is passed on. Since carbon monoxide produces a respiratory defect, and is produced during respiratory deficiency, it is one of the factors which can account for the "heritability" of cancer. When cancer cells are implanted into a healthy host, they seldom gorw, but when implanted into a host who already has cancer, they grow. (Hakim, 1988.) The healthy host provides many restorative factors, the sick host provides additional harmful factors, but few restorative factors (Chekulaev, et al., 1987.)

*(*Porphyrins are the pigment molecules that include the heme of hemoglobin, of the respiratory cytochromes, the peroxidases including thyroid peroxidase, and of the P-450 enzymes which, among other things, produce steroid hormones.)*

One of the mysteries of public health is that people who drink alcohol live longer and are generally healthier than people who don't. 30 years ago, when I began studying Warburg's work, I heard about a man with inoperable cancer who, because of his hopeless condition, began living almost exclusively on hard liquor. Aftrer six months his doctor wondered why he was still alive, and a new operation revealed that there was no cancer.

In Warburg's view, cancer has a unique requirement for glucose, because of its "respiratory defect." The ability of cancer to consume large amounts of glucose means that the body interprets cancer as stress, since the waste of glucose is the key feature of stress. The body's response to decreasing glucose is to produce larger amounts of adrenal hormones, especially cortisol. Cortisol raises the blood glucose supply by converting tissue proteins into sugar. This hormonal response to the sugar appetite of cancer is the main cause of the wasting syndrome which makes cancer so serious, and which destroys the thymus gland, causing the immune system to lose its guidance. V. S. Shapot's work, showing the involvment of glucose and cortisol in cancer, provides part of the context for understanding the implications of Warburg's view of cancer as a "respiratory defect."

Alcohol has the ability to lower blood glucose, but it does it partly by suppressing the stress-induced secretion of cortisol, reducing the conversion of the body's proteins into glucose, and shifting metabolism toward fat as an energy source. Additionally, ethyl alcohol happens to detoxify carbon monoxide, "desorbing" it from the heme groups, somewhat as light does. These properties of alcohol have been studied in hospitalized patients. In terminal cancer patients, it was found to be extremely effective in protecting against the tissue wasting process. Even in alcoholics with liver cirrhosis, it was found to be protective to the liver, when added to a good diet.

Ordinary stress lowers blood sugar and produces lactic acid, because it uses energy faster than cells can produce it by way of efficient oxidative metabolism, and so sugar is inefficiently converted to lactic acid. This form of metabolism raises the cell's internal pH, intensifies its negative electrical charge, and causes it to take up water, and to alter its control of salts and other solutes; these changes may stimulate cell division, but they can also cause cell death, or adaptive modifications.

While the genetic, the metabolic, and the adaptive processes seem to be on very different "levels," if we are to think about them in realistic and productive ways, it's necessary to follow the ways in which they are integrated, and to see *gene regulation and metabolic responses to the environment as a continuous physical-chemical process.* The myth of the isolated immortal germline, and its derivative, the "Central Dogma" of molecular genetics, that sees information flowing only from the genes into the proteins, have kept most researchers from looking elsewhere for regulatory principles.

The idea of inheritance as a rigidly fixed system has made it easy to think of biology in terms of the "lock and key" analogy, in which an enzyme and its substrate, an antibody and its antigen, an organism and its niche, a gene and its inducer, etc., have a specific, fixed, mechanical relationship. However, even the simplest organisms have qualities that can seem more like a locksmith who with a little time can figure out how to open locks without a key. All biological systems are simultaneously regulated on more than one level, in a coherent and unified way.

The cell's framework, the "cytoskeleton," was demonstrated early in the 20th century, but the dogmatic view of the cell as "liquid enclosed by a membrane" caused it to be ignored by most biologists until the 1970s. The cytoskeleton is strongly influenced by respiratory distress and the hormones that are so closely associated with oxidative processes, estrogen and the porphyrin derivatives in particular.

The cytoskeleton includes a variety of filaments and tubelike structures. The microtubules make up the apparatus that separates the chromosomes during cell division. Estrogen can cause almost instantaneous changes in the cytoskeleton proteins and their interations with

have a variety of actions that tend to improve respiration.

Things that protect the bowel, such as raw carrots, have far-reaching effects on hormones and immunity. Eggs contain many protective and therapeutic factors, in spite of their excess of toxic unsaturated fats resulting from an unnatural diet; egg lecithin, for example, isn't immunosuppressive, as soybean lecithin is.

The last experiments of Leonell Strong, in which he found that certain combinations of nucleosides could cause the disappearance of cancer from the progeny of the treated animals, should be taken seriously. They relate to the heritable effects of hormone balance ("hormonal imprinting"), and so have implications far beyond the prevention of cancer.

REFERENCES

Cancer 1988 Feb 15;61(4):689-701 **Peripheral blood lymphocytes from patients with cancer lack interleukin-2 receptors.**Hakim AA Department of Medicine, Stritch School of Medicine, Loyola University Medical Center, Maywood, Illinois. **When tumor cells develop in healthy adults, they activate the cellular immune system--natural killer (NK) cells, antigen-specific cytotoxic lymphocytes (CTL), and the synthesis of antigen specific cytotoxic antibodies. These are aimed at killing the intruding cells. However, in cancer patients the tumor continues to grow.** As tumor cells proliferate, they were shown to release factors that mediate the inactivation of the host immune defense systems. The study documented in this article examined peripheral blood lymphocytes, mononuclear cells (MNC), NK cells, T-helper cells (THC). This study confirmed the interaction of the released inhibitor factors with these mononuclear cells." "An elevation in the expression of cell surface antigen GP-120 has been observed to be associated with the activation in vitro of T-cells from healthy adults and from patients with benign breast disease, but not of T-cells from patients with breast carcinoma."

Endocrinology 1996 Nov;137(11):4536-41 **The cellular protooncogenes c-fos and egr-1 are regulated by prostacyclin in rodent osteoblasts and fibroblasts.** Glantschnig H, Varga F, Klaushofer K PGs are local regulators of various cellular functions. They exert their effects via specific PG receptor subtypes. **Induction of c-fos gene expression has been described for arachidonic acid and its metabolite PGE2.** We demonstrate that another very short half-lifed prostanoid metabolite, namely prostacyclin (PGI2), is a regulator of immediate-early genes. PGI2 transiently induced the growth-associated immediate-early genes c-fos and egr-1 in osteoblastic as well as fibroblastic cell lines. Furthermore, we showed that **PGI2 dose dependently stimulated new DNA synthesis in the osteoblastic cell line MC3T3-E1.** Although PGI2 is known to be a potent inducer of cyclooxygenases, we showed that this **pathway is not necessary for protooncogene induction by PGI2. Our data indicate a direct effect of PGI2 on immediate-early gene** expression, which does not depend on the synthesis of other prostanoids. Intracellular signal transduction mechanisms were studied with the protein kinase inhibitor H-7, a potent inhibitor of PGI2-induced c-fos expression. Experiments with phorbol esters revealed that protein kinase C activity is not obligatory for the effect of PGI2 on c-fos expression. We conclude from these results that PGI2, a rapidly inactivated prostanoid, has a major impact on cellular oncogene expression and growth in mesenchymally derived cells.

Proc Natl Acad Sci U S A 1998 Nov 10;95(23):13692-7 **Genetic instability of cancer cells is proportional to their degree of aneuploidy.** Duesberg P, Rausch C, Rasnick D, Hehlmann R III Genetic and phenotypic instability are hallmarks of cancer cells, but their cause is not clear. The leading hypothesis suggests that a poorly defined gene mutation generates genetic instability and that some of many subsequent mutations then cause cancer. Here we investigate the hypothesis that genetic instability of cancer cells is caused by aneuploidy, an abnormal balance of chromosomes. Because symmetrical segregation of chromosomes depends on exactly two copies of mitosis genes, aneuploidy involving chromosomes with mitosis genes will destabilize the karyotype. The hypothesis predicts that the degree of genetic instability should be proportional to the degree of aneuploidy. Thus it should be difficult, if not impossible, to maintain the particular karyotype of a highly aneuploid cancer cell on clonal propagation. This prediction was confirmed with clonal cultures of chemically transformed, aneuploid Chinese hamster embryo cells. It was found that the higher the ploidy factor of a clone, the more unstable was its karyotype. The ploidy factor is the quotient of the modal chromosome number divided by the normal number of the species. Transformed Chinese hamster embryo cells with a ploidy factor of 1.7 were estimated to change their karyotype at a rate of about 3% per generation, compared with 1.8% for cells with a ploidy factor of 0.95. Because the background noise of karyotyping is relatively high, the cells with low ploidy factor may be more stable than our method suggests. The karyotype instability of human colon cancer cell lines, recently analyzed by Lengnauer et al. [Lengnauer, C., Kinzler, K. W. & Vogelstein, B. (1997) Nature (London) 386, 623-627], also corresponds exactly to their degree of aneuploidy. We conclude that aneuploidy is sufficient to explain genetic instability and the resulting karyotypic and phenotypic heterogeneity of cancer cells, **independent of gene mutation.** Because aneuploidy has also been proposed to cause cancer, our hypothesis offers a common, unique mechanism of altering

water. Under the influence of estrogen, microtubules, for example, change their physical state and appear to dissolve. Experiments show that increasing amounts of estrogen cause progressively more severe disruptions of the mitotic apparatus, producing lopsided cell divisions. (When these abnormal "mitotic figures" are found in breast tissue, it means that cells are being killed by an excess of estrogen, but these dying cells are usually taken as "evidence of cancer.")

Biliverdin, produced along with carbon monoxide in the breakdown of heme, causes changes in other parts of the cytoskeleton, including the proteins in the nucleus which help to regulate the movements of nucleic acids.

Carbon monoxide, and other substances such as nitric oxide which also function as respiratory poisons, suppress energy production by the mitochondria, and this activates enzymes which cut DNA molecules, producing either DNA rearrangement, or apoptotic cell death.

All of these well established observations show the steps by which physiological reactions can lead to chromosomal and genetic abnormalities.

The steps toward cancer include chronic irritation, recognized for centuries as a cause of cancer; an inflammatory reaction activates enzymes that release estrogen. (Histamine, free fatty acids, prostaglandins, calcium and other products of inflammation are further promoted by estrogen, and synergize with it.)

Estrogen activates other inflammatory "acute phase" and stress-shock proteins, including heme oxygenase, which produces carbon monoxide and biliverdin. These are powerful protective and antioxidant factors making cells resistant to apoptosis and regulation.

Biliverdin (F. Paradisi, 1975) activates beta-glucuronidase, one of the main enzymes involved in the local release of estrogen.

In later stages, anemia and hemolysis are associated with CO and biliverdin production from the release of heme. (Anemia is seen in most patients with advanced cancer.) Biliverdin, combined with estrogen and hypoxia, alters the cytoskeleton, producing chromosome imbalances, causing mutations and activating the "oncogenes."

The respiratory defect of cancer interferes with the production of carbon dioxide. CO2 suppresses lactic acid formation, and is essential for all normal cell processes; it appears to be an important factor in producing cellular differentiation (Dictyostelium).

Estrogen promotes lactic acid formation, and promotes porphyrin synthesis, providing the material for forming heme and carbon monoxide. Besides causing porphyria, estrogen causes many other liver diseases, including cholestasis, the failure to release bile; the inhibition of carbon monoxide formation has been found to promote bile flow. Estrogen promotes the formation of the enzyme heme oxygenase, which forms carbon monoxide from heme.

Carbon monoxide increases the formation of cortisol, by stimulating ACTH release from the pituitary.

The stress response is self-sustaining on several levels. For example, stress increases the absorption of bacterial endotoxin from the intestine, which increases the estrogen level and synergizes with biliverdin and cortisol.

While estrogen does cause direct DNA damage, its clearest effect in carcinogenesis is on the cytoskeleton which regulates cell division, and defective cell division, rather than "gene mutation," is one of the important steps in the progression of cancer (Rubin, Duesberg).

Cancer cells are protected against the body's ability to destroy them, by the antioxidant functions of carbon dioxide and biliverdin..

The multilevel physiological nature of cancer calls for a multilevel physiological approach to its prevention and cure.

This would include antiestrogen regimes, antiinflammatory and antihistamine factors (histamine interacts closely with nitric oxide and carbon monoxide), adequate nutrition, carbon dioxide, and specific anti-carbon monoxide therapies (such as light, alcohol, and possibly the minerals which convert porphyrin into compounds that inhibit the production of carbon monoxide), and methods to decrease nitric oxide formation and to restrain cortisol production, since these promote the formation of carbon monoxide. **One of the most interesting approaches to inhibiting carbon monoxide production is to use vitamin B12, as hydroxocobalamin, as an antidote to nitric oxide, preventing the nitric oxide from stimulating the formation of heme oxygenase.** Wherever carbon monoxide mediates a biological malfunction, as in aquired immunodeficiency, Alzheimer's disease, and cancer, vitamin B12 seems to have a place as a detoxicant.

Progesterone and thyroid have several desirable properties that make them generally useful in cancer prevention and therapy. The shorter-chain saturated fatty acids contained in coconut oil have several beneficial effects. Aspirin and vitamin E

and simultaneously destabilizing normal cellular phenotypes.

Proc Natl Acad Sci U S A 1997 Dec 23;94(26):14506-11 **Aneuploidy correlated 100% with chemical transformation of Chinese hamster cells.** Li R, Yerganian G, Duesberg P, Kraemer A, Willer A, Rausch C, Hehlmann R Aneuploidy or chromosome imbalance is the most massive genetic abnormality of cancer cells. It used to be considered the cause of cancer when it was discovered more than 100 years ago. Since the discovery of the **gene,** the aneuploidy hypothesis has lost ground to the hypothesis that mutation of cellular genes causes cancer. According to this hypothesis, cancers are diploid and aneuploidy is secondary or nonessential. Here we reexamine the aneuploidy hypothesis in view of the fact that nearly all solid cancers are aneuploid, **that many carcinogens are nongenotoxic,** and that mutated genes from cancer cells **do not transform diploid human or animal cells.** By regrouping the gene pool-as in speciation-aneuploidy inevitably will alter many genetic programs. This genetic revolution can explain the numerous unique properties of cancer cells, such as invasiveness, dedifferentiation, distinct morphology, and specific surface antigens, much better than gene mutation, which is limited by the conservation of the existing chromosome structure. To determine whether aneuploidy is a cause or a consequence of transformation, we have analyzed the chromosomes of Chinese hamster embryo (CHE) cells transformed in vitro. This system allows (i) detection of transformation within 2 months and thus about 5 months sooner than carcinogenesis and (ii) the generation of many more transformants per cost than carcinogenesis. To minimize mutation of cellular genes, we have used nongenotoxic carcinogens. **It was found that 44 out of 44 colonies of CHE cells transformed by benz[a]pyrene, methylcholanthrene, dimethylbenzanthracene, and colcemid, or spontaneously were between 50 and 100% aneuploid.** Thus, aneuploidy originated with transformation. Two of two chemically transformed colonies tested were tumorigenic 2 months after inoculation into hamsters. The cells of transformed colonies were heterogeneous in chromosome number, consistent with the hypothesis that aneuploidy can perpetually destabilize the chromosome number because it unbalances the elements of the mitotic apparatus. Considering that all 44 transformed colonies analyzed were aneuploid, and the early association between aneuploidy, transformation, and tumorigenicity, we conclude that aneuploidy is the cause rather than a consequence of transformation.

Proc Natl Acad Sci U S A 1997 Sep 2;94(18):9614-9 **Dominant transformation by mutated human ras genes in vitro requires more than 100 times higher expression than is observed in cancers.** Hua VY, Wang WK, Duesberg PH The gene-mutation-cancer hypothesis holds that mutated cellular protooncogenes, such as point-mutated proto-ras, "play a dominant part in cancer," because they are sufficient to transform transfected mouse cell lines in vitro [Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1994) Molecular Biology of the Cell (Garland, New York)]. However, in cells transformed in vitro mutated human ras genes are expressed more than 100-fold than in the cancers from which they are isolated. In **view of the discrepancy between the very low levels of ras transcription in cancers and the very high levels in cells transformed in vitro,** we have investigated the minimal level of human ras expression for transformation in vitro. Using point-mutated human ras genes recombined with different promoters from **either human metallothionein-IIA or human** fibronectin or from retroviruses we found dominant in vitro transformation of the mouse C3H cell line only with ras genes linked to viral promoters. These ras genes were expressed more than 120-fold higher than are native ras genes of C3H cells. The copy number of transfected ras genes ranged from 2-6 in our system. In addition, nondominant transformation was observed in a small percentage (2-7%) of C3H cells transfected with ras genes that are expressed less than 20 times higher than native C3H ras genes. Because over 90% of cells expressing ras at this moderately enhanced level were untransformed, **transformation must follow either a nondominant ras mechanism or a non-ras mechanism.** We conclude that the mutated, but normally expressed, ras genes found in human and animal cancers are not likely to "play a dominant part in cancer." The conclusion that mutated ras genes are **not sufficient or dominant** for cancer is directly supported by recent discoveries of **mutated ras in normal animals, and in benign human tissue, "which has little potential to progress" [Jen, J., Powell, S. M., Papadopoulos, N., Smith, K. J., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1994) Cancer Res. 54, 5523-5526]. Even the view that mutated ras is necessary for cancer is hard to reconcile with (i) otherwise indistinguishable cancers with and without** ras mutations, (ii) metastases of the same human cancers with and without ras mutations, (iii) retroviral ras genes that are oncogenic without point mutations, and (iv) human tumor cells having spontaneously lost ras mutation but not tumorigencity.

Proc Natl Acad Sci U S A 1990 Dec;87(24):10005-10009 **Physiological induction and reversal of focus formation and tumorigenicity in NIH 3T3 cells.** Rubin AL, Arnstein P, Rubin H. NIH 3T3 cells undergo morphological transformation in response to conditions of constrained growth, such as occur in low serum concentrations or at confluence. Transformation is expressed in a small fraction of the cells by the appearance of discrete foci of multiplying cells on a confluent monolayer of quiescent cells. We isolated and expanded cell populations from three dense and three light foci. Cells from each of these populations efficiently reproduced foci of the same morphotype when grown on a background of nontransformed NIH 3T3 cells. Using cultures derived from one of the dense foci (subline D/2), we found that the number of focus-forming units was stable and the cells remained tumorigenic when they were subjected to repeated thrice-weekly passage in 2% calf serum. However, equivalent passage in 10% calf serum

eventually rendered the cells incapable of both focus production and tumor formation. **The results show that the capacity to produce tumors as well as morphological transformation are produced as a response to physiological constraints of growth and/or metabolism in the absence of carcinogens and that both properties can be reversed by lifting the constraints. This behavior is typical of an adaptational response and, taken together with other supporting evidence, shows that tumorigenesis does not require conventional genetic alteration.** Published erratum appears in Proc Natl Acad Sci U S A 1991 Apr 15;88(8):3510

J Surg Oncol 1974;6(3):191-195 **The transitory nature of a transmissible entity controlling the growth of a spontaneous tumor in mice.** Strong LC, Matsunaga H

J Surg Oncol 1974;6(1):29-38. **"Studies on the effects of adenosine upon tumor growth in mice."** Strong, L.C., Ishmael D.

Cytobios 1973 Jul;7(28):253-257 **Effect of 5-methyl-cytidine on growth and regression of spontaneous tumours in mice.** Strong LC, Matsunaga H

J Surg Oncol 1973;5(2):181-188 **Comparative effect of three nucleosides on suppression of cancer growth in mice.** Strong LC, Matsunaga H

Cytobios 1975;12(45):13-18 **An inverse correlation (dose-response between 5-methyl-cytidine and the fate of spontaneous tumours in mice.** Strong LC, Matsunaga H Tumour-bearing mice, 248 in number, have been injected with 5-methyl-cytidine dissolved in physiological salt solution. The mice were divided into 5 series depending upon the amount of 5-methyl-cytidine they received. Mice of a successive series received 43.2, 28.8, 14.4 and 7.2 mg/kg body weight. There was a progressive increase in the percentage of mice showing regression of tumours in the successive series, as follows: 45%, 50%, 65%, 60%, and 67.8% respectively, giving an average percentage of regressions of 59.7%. The incidence of mice developing multiple primary tumours increased in the same successive series respectively as follows: 25%, 43.8%, 58.3%, 73.3%, and 70%, thus giving an average of 58.3%. The evidence obtained indicates an inverse correlation (dose-response) between the amount of the 5-methyl-cytidine injected and the percentage of regressions of spontaneous tumours.

J Surg Oncol 1975;7(5):367-373 **Further studies on a transmissible entity in relation to the control of cancer in mice.** Strong LC, Matsunaga H Tumor-bearing mice of the C3H/ST inbreds were used in this investigation. Of the 598 mice, 290 were controls, 248 received injections of 5-methyl cytidine dissolved in physiological salt solution, and 60 received injections of three nucleosides - adenosine, 6-methyl adenosine, and 5-methyl cytidine - also dissolved in physiological salt solution. The mice were divided into 6 groups based upon the number of generations of descent from a mouse injected with an alcohol-soluble liver extract. The criterion of tumor growth is the average increment of growth at the 20th observation period in the 7th week (3 per week). There is a cumulative inhibition of tumor growth, compared with controls, when 5-methyl cytidine is added to the effect of a transmissible entity which occurs in the lineal descendants of a tumor-bearing mouse injected with an alcohol-soluble liver extract and when the combination of three nucleosides in the same ratio of molar concentrations as in the liver extract is added to the effect of the transmissible entity.

J Surg Oncol 1975;7(6):531-534 **An inverse dose response of 5-methyl cytidine on multiple primary spontaneous tumors and their regressions in mice.** Strong LC, Matsunaga H Ten series mice of C3H/ST inbreds with spontaneous tumors have received 5-methyl cytidine (total 500 mice). Graded single doses of (1) 3.86, (2) 2.57, (3) 2.06, (4) 1.54, (5) 1.03, (6) 1.54, (7) 1.03, (8) 0.51, (9) 0.26, and (10) 0.13 mg/injection (three times weekly) were used. In series 1-5 the 5-methyl cytidine was dissolved in distilled water, and in series 6-10, the nucleoside was dissolved in physiological salt solution. In doses administered, series 4-5 overlapped with series 6-7. The number of mice that showed a regression of tumor increased in the successive series (48%-63% in series 1-5 and 25%-73% in series 6-10). As the number of mice with regressing tumors increased, the percentage of mice showing multiple primary tumors also increased. The administration of the nucleoside in physiological salt solution altered the inhibitory effect of 5-methyl cytidine on spontaneous tumors. For example, in physiological salt solution the 0.51 mg/injection gave as much inhibitory action as 1.03 mg/injection in distilled water. An inverse dose response is thus indicated between the amount of 5-methyl cytidine injected and the inhibition of spontaneous tumors in mice (percentage of regression and number of multiple primary spontaneous tumors are the criteria of inhibition considered).

Cytobios 1976;17(67-68):177-181 **A second method for induction of a transmissible entity in the control of cancer in mice.** Strong LC, Matsunaga H Spontaneous tumour-bearing mice of the C3H/St and subline C3HB/St inbred strains received injections of three nucleosides, adenosine, 6-methyl adenosine, and 5-methyl cytidine, prepared in the **same molecular concentration as they had occurred in an alcohol-soluble liver extract which in previous work had suppressed tumour growth.** Two transmissible entities appear to be present and they show different degrees of effect upon the growth and regression of spontaneous tumours of mammary gland origin (adenocarcinoma). The first transmissible entity (TE) occurred in the lineal descent of mice following injection of the liver extract. The second entity (TE2) appeared following injection of 5-methyl cytidine. TE manifested its maximum effect in suppressing cancer in mice during the 20th generation of a lineal descent following the injection of the original liver

extract into a mother of the cancer proband. TE2 appears to be optimal in controlling cancer in the F11 generation of a second lineal descent derived from an original cancer proband of the C3H/St inbred strain injected with 5-methyl cytidine.

J Surg Oncol 1977;9(1):99-103 **Increased effect of a transmissible entity on the control of cancer in C3 H/St mice.** Strong LC, Matsunaga HThree hundred and seventy-one mice of the C3H/St inbreds bearing spontaneous tumors of mammary gland origin have been used in this experiment. All mice studied were lineal descendents of a tumor-bearing mouse which had been injected with a liver extract. In this descent there appears to be a transmissible entity presumably induced by the injection of the liver extract. Following 15 generations of inbreeding after the appearance of a transmissible entity, a second sudden reversal of effect on cancer growth in mice has been indicated. This change has progressively altered the growth of cancer from a very low effect to a maximal effect, i.e., to a complete regression of a high percentage of the tumor. The maximal effect of the transmissible entity is the complete suppression of the growth of cancer during, at least, through the 25th period of observation, thus producing negative values of tumor growth. All these effects of the growth and fate of spontaneous tumors of mammary gland origin in mice have been obtained in a single lineal descent of C3H/St inbreds without resorting to any outcross.

J Surg Oncol 1972;4(3):248-254 **Comparison of the effect of three nucleosides and a liver emulsion upon the inhibition of cancer in mice.** Strong LC, Matsunaga H.

J Surg Oncol 1972;4(5):528-532 **5-Methyl-cytidine as an inhibitor of spontaneous cancer in mice.** Strong LC, Matsunaga H.

Z Krebsforsch Klin Onkol Cancer Res Clin Oncol 1971 Feb 26;75(3):225-228 **What is a control for cancer in mice?** Strong LC, Matsunaga H.

J Surg Oncol 1971;3(4):467-473 **The stimulation and inhibition of the growth capacities of spontaneous tumors of mammary gland origin in mice (adenocarcinomata).** Strong LC, Matsunaga H.

J Surg Oncol 1971;3(4):369-378 **The effect of 'aging' of an inhibitor of spontaneous tumors in mice.** II. Observations at aging from 101-1346 days. Strong LC, Matsunaga H.

J Surg Oncol 1970;2(4):363-372 **A "transmissible entity" in the control of cancer in mice.** Strong LC, Matsunaga H.

Z Krebsforsch Klin Onkol Cancer Res Clin Oncol 1969;72(1):32-35 **Studies of the regression of spontaneous adenocarcinomata in mice.** Strong, L.C.

Nature 1968 Oct 19;220(164):282-283 **Effect of neomycin on an inhibitor of spontaneous tumors in mice.** Strong, L.C.

Lav Ist Anat Istol Patol Perugia 1966;26(1):5-13 **Why I originated the inbred strains of mice.Strong LC.**

Am J Physiol 1997 May;272(5 Pt 1):G1268-75. **Endogenous carbon monoxide suppression stimulates bile acid-dependent biliary transport in perfused rat liver.** Sano T, Shiomi M, Wakabayashi Y, Shinoda Y, Goda N, Yamaguchi T, Nimura Y, Ishimura Y, Suematsu M This study aimed to investigate whether carbon monoxide (CO), a product of heme oxygenase that degrades protoheme IX, serves as an endogenous modulator for biliary transport. To that end, effects of zinc protoporphyrin IX (ZnPP), a heme oxygenase inhibitor, on the biliary transport were tested in perfused rat liver. Perfusion of 1 microM ZnPP abolished detectable levels of CO in the venous perfusate and increased bile acid-dependent bile output accompanying an increased secretion of bile salts. The ZnPP-induced choleresis coincided with a reduction of tissue guanosine 3',5'-cyclic monophosphate (cGMP) levels and a decrease in vascular conductance. On administration of 2.5 microM CO, ZnPP-elicited choleresis, decreases in vascular conductance, and cGMP levels were all attenuated. Treatment with 1 microM 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) partly attenuated the **ZnPP-induced choleresis** in concert with repression of vascular conductance. Furthermore, treatment of the liver with methylene blue, a guanylate cyclase inhibitor, evoked a choleresis similar to that induced by ZnPP. **Thus endogenous CO suppression stimulates the biliary transport in part through a cGMP-dependent mechanism.**

Biochem Soc Trans 1997 Aug;25(3):406S **Does carbon monoxide inhibit cytochrome oxidase in vivo?** Davies NA, Trikkas C, Cooper CE.

Urology 1996 May;47(5):727-33 **Expression of heme oxygenase-1 (HSP32) in human prostate: normal, hyperplastic, and tumor tissue distribution.** Maines MD, Abrahamsson PA OBJECTIVES. Heme oxygenase isozymes, HO-1 and HO-2, are members of the stress/heat shock (HSP) family of proteins, with the known function of cleaving the heme molecule to biliverdin, iron, and carbon monoxide. The aim of this study was to examine the pattern of tissue expression of HO-1 in the humanprostate under different states of proliferation and differentiation and to investigate whether the pattern differs between these states. METHODS. Presently, we have determined the pattern of tissue expression of the stress-inducible isozyme, HO-1 (HSP32), in human prostate under normal and pathologic conditions, by immunohistochemistry, using polyclonal antibodies, and have measured HO-1 and HO-2 mRNA levels in normal prostate and benign prostatic hyperplasia (BPH) by Northern blotting. The activity of prostate to

catalyze heme degradation was also assessed. RESULTS. In normal and BPH tissue, columnar epithelial cells of acini and ducts and cells in stroma displayed HO-1 immunoreactivity; in all cells, perinuclear staining was prominent. In BPH tissue, however, a more intense staining of the epithelial cells occurred, with notable staining of the basal cells. In undifferentiated malignant tumors, intense HO-1 staining was manifest in nearly all tumor cells, and also in the epithelial lining of blood vessels. HO-1 in the prostate tissue was found catalytically active and oxidatively cleaved the heme molecule (Fe-protoporphyrin IX) to biliverdin. Northern blot analysis shows that two forms of HO are present in the human prostate. Compared with normal tissue, predominantly hyperplastic tissue demonstrates a pronounced increase in the approximately 1.8 kb mRNA that hybridizes to the rat HO-1 probe. The levels of two transcripts, approximately 1.3 and approximately 1.7 kb, that hybridize to the rat HO-2 probe are not increased in BPH tissue. CONCLUSIONS. **The finding that HO-1 expression is increased in BPH and malignant prostate tissue is consistent with a role for this stress protein in the pathogenesis of BPH and prostate cancer; in the context of iron metabolism, an argument is made in support of this possibility.**

Sangyo Igaku 1990 Jan;32(1):33-40 **[Effects of lead on drug metabolizing enzymes, cytochrome P-450 and hemeoxygenase in rats].** [Article in Japanese] Harada K, Ohmori S, Nagano M, Miura H "In a supplementary experiment, lead was found to decrease the contents of heme in the **microsome and to increase the activity of hemeoxygenase. "**

Cancer Res 1991 Feb 1;51(3):974-8 **Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells.** Applegate LA, Luscher P, Tyrrell RM. Accumulation of heme oxygenase mRNA is strongly stimulated by treatment of cultured human skin **fibroblasts with ultraviolet radiation, hydrogen peroxide, or the sulfhydryl reagent sodium arsenite (S. M.** Keyse and R. M. Tyrrell. Proc. Natl. Acad. Sci. USA, 86: 99-103, 1989). Since this will result in a transient reduction in the prooxidant state of cells, the phenomenon may represent an important inducible antioxidant defense mechanism. To examine the generality of the response, we have measured the accumulation of the specific mRNA in a variety of human and mammalian cell types after inducing treatments. Induction by sodium arsenite is observed in all additional human cell types tested. This includes primary epidermal keratinocytes and lung and colon fibroblasts as well as established cell lines such as HeLa, TK6 lymphoblastoid, and transformed fetal keratinocytes. Strong induction of heme oxygenase mRNA is also observed following sodium arsenite treatment of cell lines of rat, hamster, mouse, monkey, and marsupial origin. The agents which lead to induction in cultured human skin fibroblasts fall into two categories: (a) those which are oxidants or can generate active intermediates (ultraviolet A radiation, hydrogen peroxide, menadione, and the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate); (b) agents which are known to interact with or modify cellular glutathione levels (buthionine sulfoximine, sodium arsenite, iodoacetamide, diamide, and cadmium chloride). These observations strongly support the hypothesis that induction of the enzyme is a general response to oxidant stress in mammalian cells and are consistent with the possibility that the cellular redox state plays a key role.

Carcinogenesis 1992 Feb;13(2):227-32 **Endogenous glutathione levels modulate both constitutive and UVA radiation/hydrogen peroxide inducible expression of the human heme oxygenase gene.** Lautier D, Luscher P, Tyrrell RM Swiss Induction of the expression of the mammalian heme oxygenase gene appears to be a general response to oxidant stress. In view of the role of glutathione in protecting cells against solar UVA radiation and other forms of oxidant stress, we have investigated the relationship between intracellular glutathione levels and the inducibility of the human heme oxygenase gene after treatment of populations of cultured skin fibroblasts with either UVA radiation or hydrogen peroxide. We observe a clear relationship between cellular glutathione status and both the constitutive and oxidant-inducible accumulation of heme oxygenase mRNA. Glutathione depletion may lead to enhanced gene expression either as a result of the potentiated accumulation of active oxygen intermediates or as a result of the direct influence of glutathione on a critical target involved in signal transduction.

Bull Cancer 1990;77(5):479-83 **[Modification of the cellular cytoskeleton and hepatic tumor promotion].** [Article in French] Decloitre F, Martin M, Ouldelhkim M, Nizard C. Non transformed epithelial hepatic cells (established cell line and adult rat hepatocytes) treated by liver **tumor promoters, phenobarbital and biliverdin,** for 24 and 48 h showed a fragmentation and loss of F-actin and a depolymerisation of microtubules. This pattern closely resembles that of transformed cells which were not susceptible to the action of promoters. In liver preneoplastic nodules obtained from rats submitted to an initiation-promotion process, actin almost completely disappeared with the concomitant appearance of a characteristic enzymatic pattern rich in GGT and GST-P. Therefore, cytoskeleton of hepatic cells is a target for tumor promoters and could play a role in promotion mechanism.

J Biochem (Tokyo) 1997 Jun;121(6):1162-8 **Possible implications of the induction of human heme oxygenase-1 by nitric oxide donors.** Takahashi K, Hara E, Ogawa K, Kimura D, Fujita H, Shibahara S. To explore the involvement of nitric oxide (NO) in the induction of heme oxygenase-1, an essential enzyme in heme catabolism, we studied the effects of NO donors on the expression of heme oxygenase-1 **mRNA in HeLa human cervical cancer cells. Treatment with each of three NO donors, sodium nitroprusside, 3-morpholinosydnonimine, and S-nitroso-L-glutathione, caused noticeable increases in**

the expression levels of heme oxygenase- mRNA, but not heme oxygenase-2 mRNA. On the other hand, nitrite or 8-bromo cGMP exerted no noticeable effect on the levels of heme oxygenase-1 mRNA. We showed that sodium nitroprusside also increased the **levels of heme oxygenase-1 protein.** The sodium nitroprusside-mediated increase in heme oxygenase-1 mRNA levels was abolished by treatment with actinomycin D. The expression levels of heme oxygenase-1 mRNA were also **increased by NO donors in human melanoma and neuroblastoma cell lines.** Thus, the observed induction of heme oxygenase-1 may represent an important response to NO or NO-related **oxidative stress.** The half lives of heme oxygenase-1 and heme oxygenase-2 mRNAs were estimated to be about 3.2 h and more than 5 h, respectively.

Ann N Y Acad Sci 1998 May 1;840:249-61 **Acute and subacute effects of endotoxin on hypothalamic gaseous neuromodulators.** Kostoglou-Athanassiou I, Jacobs RA, Satta MA, Dahia PL, Costa A, Navarra P, Chew SL, Forsling ML, Grossman, A.B.

Steroids, 1998, 63(5-6):252-6, **Neuroendocrine mechanisms underlying the control of gonadotropin secretion by steroids.** Mahesh VB, Brann DW. There is considerable evidence that although estradiol may trigger the preovulatory surge of gonadotropins, progesterone is required for its full magnitude and duration and that glucocorticoids bring about selective follicle-stimulating hormone release. The luteinizing hormone-releasing hormone (LHRH) neuron does not have steroid receptors and is regulated by excitatory amino acid neurotransmission. **Steroids do not appear to modulate excitatory amino acid receptors directly but increase release of glutamate in the preoptic area. This may be due to the suppression by steroids** of the enzyme glutamatic acid decarboxylase67 that converts glutamate into GABA. NMDA receptors colocalize with nitric oxide synthase-containing neurons that surround the LHRH neurons in the preoptic area and intersect the LHRH fibers in the median eminence. Other potential novel pathways of LHRH release that are currently being explored include carbon monoxide generated by the action of heme oxygenase-2 on heme molecules and bradykinin acting via bradykinin B2 receptors.

Biochem Biophys Res Commun 1997 Dec 18;241(2):215-20 **Involvement of the heme oxygenase-carbon monoxide pathway in keratinocyte proliferation.** Clark JE, Green CJ, Motterlini R. It has been suggested that nitric oxide (NO), a small gaseous molecule with a multiplicity of cellular functions, plays an important part in the regulation of cellular proliferation. We have examined the effect of the NO donor sodium nitroprusside (SNP) on heme oxygenase-1 (HO-1) expression in human **epidermal keratinocytes and investigated the contribution of the heme oxygenase pathway in the control of** keratinocyte proliferation. Incubation of keratinocytes with 0.5 mM SNP resulted in a **2.5-fold increase in heme oxygenase activity which was reflected by a significant increase in HO-1** protein expression, as measured by Western blot. This effect was associated with a **200% increase in keratinocyte proliferation.** The proliferative effect of the NO donor was totally abolished by co-incubation of SNP with tin protoporphyrin IX, a potent inhibitor of heme oxygenase, or hydroxocobalamin, a NO scavenger. These results suggest that the heme oxygenase pathway is involved in keratinocyte proliferation mediated by NO.

J Neuroimmunol 1998 Jun 1;86(1):104-9 **Endotoxin stimulates an endogenous pathway regulating corticotropin-releasing hormone and vasopressin release involving the generation of nitric oxide and carbon monoxide.** Kostoglou-Athanassiou I, Costa A, Navarra P, Nappi G, Forsling ML, Grossman AB.

Ann N Y Acad Sci 1998 May 1;840:249-61 **Acute and subacute effects of endotoxin on hypothalamic gaseous neuromodulators.** Kostoglou-Athanassiou I, Jacobs RA, Satta MA, Dahia PL, Costa A, Navarra P, Chew SL, Forsling ML, Grossman AB.

Cell Biol Toxicol 1990 Jan;6(1):23-34 **Modifications of microfilaments and microtubules induced by two hepatic tumor promoters, phenobarbital and biliverdin in non-transformed and transformed hepatic cell lines.** Decloitre F, Lafarge-Frayssinet C, Martin M, Frayssinet C. "Microfilaments and microtubules are components of the cytoskeleton which could be implicated in neoplastic transformation. We studied the effect of **two hepatic tumor promoters, phenobarbital (PB) and biliverdin** (BV), on microfilaments and microtubules of non-transformed (Cl3) and transformed (FV) hepatic epithelial cells. Cl3 non-transformed cells cultured in the presence of I X 10(-6) M BV for 48 h showed a loss of F-actin, fragmentation of actin and the appearance of star-like structures in the cytoplasm, as well as loosening of the peripheral bundle of actin, and some ruffling of cell membranes." "BV and PB also produced in these cells modifications of microtubules characterized by a disappearance of centrosome staining in numerous cells, a condensed ring of tubulin around the nucleus and a depolymerized aspect of the microtubular network. All these modifications of microfilaments and microtubules closely resembled those observed in FV transformed cells in the absence of any treatment (Solvent DMSO only). We did not observe an effect of BV and PB on FV cells. The present data demonstrate that the cytoskeleton of non-transformed epithelial liver cells is sensitive to the action of liver tumor promoters suggesting that it might play a role as to yet be defined in the promotion mechanism. "

Bull Cancer 1990;77(5):475-8 **[Oncogenes and tumor promotion].** [Article in French] Lafarge-Frayssinet C, Frayssinet C. We have studied the effects of 2 hepatic promoters: an exogenous one:phenobarbital, and an endogenous **one biliverdin (a bile pigment) on the expression of 3 oncogenes c-Ki-ras, c-fos and c-myc involved in**

growth process, differentiation and tumorigenesis for the liver. This work was done: 1) in vitro using epithelial liver cell strains originated from 10-day old rats; 2) in vivo using regenerating liver after partial hepatectomy, as well as preneoplastic nodules obtained by the Solt and Farber procedure. In all cases we have shown an important overexpression of the oncogenes.

Cell Mol Biol 1981;27(2-3):77-82 **Enhancement of DNA synthesis by biliverdin in a non-transformed liver cell strain.** Lafarge-Frayssinet C, Morel-Chany E, Trincal G, Frayssinet C Cancer Lett 1983 Apr;18(3):277-82 **Promoting effect of biliverdin for liver cells initiated by aflatoxin B1.** Lafarge-Frayssinet C, Morel-Chany E, Trincal G, Frayssinet C In this paper we have studied the participation of biliverdin, a bile pigment produced from hemoglobin catabolism, in a two-stage carcinogenesis model using hepatic cells in culture. The initiation was realised by a short term exposure to low doses of aflatoxin B1. The cells were then cultivated in the presence of biliverdin in the medium. We observed that biliverdin increased the rate and the efficiency of neoplastic transformation of cells. **These results suggest that biliverdin may act as a promoting substance.**

Cancer Lett 1989 Mar;44(3):191-8 **Over expression of proto-oncogenes: ki-ras, fos and myc in rat liver cells treated in vitro by two liver tumor promoters: phenobarbital and biliverdin.** Lafarge-Frayssinet C, Frayssinet C Among the liver cell strains established in the laboratory from the liver of 10-day-old rats, some of them (FV) underwent spontaneous neoplastic transformation after a number of subcultures. However, one (Cl3) had maintained a non-transformed phenotype (persistence of contact inhibition, lack of growth in soft agar and of gamma glutamyltranspeptidase activity). These cells were grown either for a short time (48 h) or a longer time (3 weeks) in the presence of two liver tumor promoters: phenobarbital (0.2 x 10(-3) M) or biliverdin (10(-6) M). Total RNA was analysed by dot blot and Northern blot, then hybridized with ki-ras, fos and myc probes, previously labelled with 32P by nick translation. The three oncogenes were well expressed by the two strains but while an over-expression was observed for the Cl3 non-transformed cells when they were grown in presence of phenobarbital or biliverdin, the FV-transformed cells were not sensitive to the two promoters.

Pathol Biol (Paris) 1975 Feb;23(2):101-5 **A cytochemical study of some enzyme activities in biliverdin-treated cell cultures.** Paradisi F, Graziano L, de Ritis F The authors studied the modifications of the activities of some enzymes in cell cultures submitted to the action of biliverdin. This biliary pigment rapidly induces a **remarkable increase in alkaline phosphatase and ATP-ase activities and subsequently, an activation of acid phosphatase and beta-glucuronidase.** On the contrary, 5'-nucleotidase and glucose-6-phosphatase activities remain unchanged. These results are discussed and compared with those obtained in our and other laboratories by using unconjugated bilirubin on different biological substrates.

Mol Pharmacol 1997 Oct;52(4):590-9 **Aryl hydrocarbon receptor-dependent induction of cyp1a1 by bilirubin in mouse hepatoma hepa 1c1c7 cells.** Sinal CJ, Bend JR "Heme metabolism normally involves enzymatic conversion to biliverdin and subsequently to bilirubin, catalyzed by heme oxygenase and biliverdin reductase, respectively. We examined the ability of exogenously added hemin, biliverdin, or bilirubin to regulate Cyp1a1, an enzyme that may be active in bilirubin elimination. A substantial dose-dependent increase in Cyp1a1 mRNA occurred after treatment of Hepa 1c1c7 cells with either of the three compounds. This increase was readily apparent 1 hr after treatment with biliverdin or bilirubin but required >/=2 hr with hemin." "These data indicate that bilirubin induces Cyp1a1 gene transcription through direct interaction with the AHR. In contrast, hemin **and biliverdin seem to induce Cyp1a1 indirectly by serving as precursors to the endogenous formation of bilirubin via normal heme metabolism pathways. This is the first direct demonstration that the endogenous heme metabolite bilirubin can directly regulate Cyp1a1 gene expression and enzymatic activity in an AHR-dependent manner."**

J Biochem (Tokyo) 1997 Jun;121(6):1162-8 **Possible implications of the induction of human heme oxygenase-1 by nitric oxide donors.** Takahashi K, Hara E, Ogawa K, Kimura D, Fujita H, Shibahara S To explore the involvement of nitric oxide (NO) in the induction of heme oxygenase-1, an essential enzyme in heme catabolism, we studied the effects of NO donors on the expression of heme oxygenase-1 **mRNA in HeLa human cervical cancer cells. Treatment with each of three NO donors, sodium nitroprusside, 3-morpholinosydnonimine, and S-nitroso-L-glutathione, caused noticeable increases in the expression levels of heme oxygenase- mRNA, but** not heme oxygenase-2 mRNA. On the other hand, nitrite or 8-bromo cGMP exerted no noticeable effect on the levels of heme oxygenase-1 mRNA. We showed that sodium nitroprusside also increased the **levels of heme oxygenase-1 protein.** The sodium nitroprusside-mediated increase in heme oxygenase-1 mRNA levels was abolished by treatment with actinomycin D. The expression levels of heme oxygenase-1 mRNA were also **increased by NO donors in human melanoma and neuroblastoma cell lines.** Thus, the observed induction of heme oxygenase-1 may represent an important response to NO or NO-related **oxidative stress.** The half lives of heme oxygenase-1 and heme oxygenase-2 mRNAs were estimated to be about 3.2 h and more than 5 h, respectively.

Ann N Y Acad Sci 1998 May 1;840:249-61 **Acute and subacute effects of endotoxin on hypothalamic gaseous neuromodulators.** Kostoglou-Athanassiou I, Jacobs RA,

Satta MA, Dahia PL, Costa A, Navarra P, Chew SL, Forsling ML, Grossman AB.

Biokhimiia 1987 Sep;52(9):1501-11 **[Activation of lipolysis and ketogenesis in tumor-bearing animals as a reflection of chronic stress states].** [Article in Russian] Chekulaev VA, Shelepov VP, Pasha-zade GR, Shapot VS In order to elucidate the peculiarities of brain metabolism in tumour-bearing organisms, the arterio-venous (A-V) content of glucose, acetoacetate (Ac-Ac), beta-hydroxybutyrate (beta-HB) and non-esterified fatty acids (NEFA) in growing Zajdela ascite hepatoma (ZAH) and solid hepatoma 27 (H-27) was compared. Analysis of metabolic patterns of healthy, starving and fed recipients (ZAH and H-27) revealed the inadequacy of the concepts on anorexia as being the cause of carbohydrate-lipid metabolic disturbances. In tumour-bearing organisms **lipolysis and ketogenesis reflect the tumour-induced chronic stress.** Absorption of beta-HB and release of Ac-Ac by brain were observed at all stages of malignant growth. **This is probably due to a partial switch-over of brain metabolism to non-carbohydrate energy sources.** Besides, certain stages of tumour growth are associated with **active assimilation of NEFA by brain.** A correlation between the A-V difference with respect to glucose and Ac-Ac as well as between the glucose and NEFA contents was established. It was assumed that the A-V difference in glucose is the main regulator of ketone body metabolism.

Arkh Patol 1987;49(6):10-8 **[Combination of immunodepression and disorders in nucleic acid metabolism of lymphoid tissue as a manifestation of a paraneoplastic syndrome].** [Article in Russian] Potapova GI, Shapot VS Several physiological, biochemical, and molecular biological approaches to the study of factors determining immunodepression in tumor-bearing animals are considered. Cancer cells release substances of nucleic and peptide nature that suppress the functional activity of macrophages and lymphocytes and stimulate cell proliferation in organs and tissues of the host. Suppressor T cells capable of inhibiting the function of helper T cells and impairing the differentiation of killer T cells are activated. The suppression **of T- and B-cell-mediated immunity in the tumor host involves disturbances of nucleic acid metabolism in those cells as well as hypersecretion of glucocorticoids.** The impairments of lymphocyte proliferation and differentiation that result in reduced immune responsiveness are attributable to drastic alterations in the metabolism of purine and pyrimidine nucleotides and to the damage sustained by the lymphocyte's DNA.

Eksp Onkol 1987;9(6):62-7 **[Relation between disorders of glucose metabolism, secretion of somatotropic hormone, thyroxine, thyrotropin and hematocrit indices in rats with transplanted hepatomas].** [Article in Russian] Shelepov VP, Pasha-zade GR, Chekulaev VA, Shapot VS **The levels of growth hormone (GH) in the blood of rats with ascite Zajdela hepatoma and hepatoma 27 (H-27) are shown to increase during the tumour growth. Stimulation of the GH secretion is a result of the hypoglycaemic stress.** An increase in the blood GH secretion is also observed in the fasting rats. To reveal the **predominance of catabolic GH effects over the anabolic ones** in the tumour host determination of the molar insulin/GH ratio in the blood is suggested. A direct correlation is found between reduction of this index, glycaemia and the content of liver glycogen. A short-term induced hyperglycaemia evokes a paradoxical reaction in terms of GH secretion. In contrast to control fasting rats, in tumour-bearing animals no **correlation between thyrotropin and thyroxine blood concentration could be observed. Anaemia increasing progressively in the course of the tumour growth may be the cause of the above phenomenon.**

J Neuroendocrinol 1998 Oct;10(10):793-802 Influence of carbon monoxide, and its interaction with nitric oxide, on the adrenocorticotropin hormone response of the normal rat to a physico-emotional stress. Turnbull AV, Kim CK, Lee S, Rivier CL "We determined whether the gas carbon monoxide (CO) altered the adrenocorticotropin hormone (ACTH) response to mild inescapable electrofootshocks, and whether it interacted with nitric oxide (NO). Peripheral injection of the NO synthase (NOS) inhibitor Nomeganitro-L-arginine-methylester (L-NAME), a compound which readily crosses the blood-brain barrier, produced the expected blunting of the ACTH response to the shocks. This effect was mimicked by other arginine analogues such as L-nitroarginine (L-NNA) and NG-methyl-L-arginine (NMMA). The subcutaneous (s.c.) administration of the heme oxygenase (HO) blockers tin mesoporphyrin (SnMP) or tin protoporphyrin (SnPP) significantly decreased brain HO levels, indicating that both compounds had penetrated the brain." "SnMP and SnPP both decreased shock-induced ACTH release, though the magnitude of this effect was slightly less than that of L-NAME. The influence of SnPP was further augmented in rats with concomitant blockade of NO formation, which suggests that both NO and CO are necessary for the full response of this axis to electrofootshocks. Finally, the ability of SnPP to significantly blunt the expression of the mRNA for the immediate early gene NGFI-B in the paraventricular nucleus (PVN) of rats exposed to shocks, indicates that the influence of CO was exerted on hypothalamic neuronal activity. Collectively, our results show that NO and CO exert a **stimulatory effect on the HPA axis response to mild electrofootshocks, and that at least part of this influence takes place on hypothalamic neurons and/or their afferents."**

J Neurochem 1994 Sep;63(3):953-62 **Corticosterone regulates heme oxygenase-2 and NO synthase transcription and protein expression in rat brain.** Weber CM, Eke BC, Maines MD Heme oxygenase (HO)-1 and -2 produce carbon monoxide, which is suspected, as is nitric oxide (NO), to function as a neuronal messenger. We report on glucocorticoid-mediated modulation of HO-2 and NO synthase expression in brain and the differential response of the two proteins to corticosterone in different brain regions. Corticosterone treatment (40 mg/kg, 20 days) had opposing effects on HO-2 and NO synthase transcript levels:

increasing the 1.3- and 1.9-kb HO-2 mRNAs and decreasing that of the brain-specific 10.5-kb NO synthase. Corticosterone did not uniformly affect HO-2 protein expression in all regions, but appeared to cause a universal reduction in NO synthase, e.g., HO-2 was decreased in hippocampus (CA1 and dentate gyrus), but not in cerebellum. In contrast, NADPH diaphorase staining was reduced in hippocampus and in molecular and granule layers of cerebellum (not detected in Purkinje cells). **Striking deficits in neuronal morphology and number of diaphorase-staining neurons were observed in the lateral tegmental area, paraventricular nucleus, and frontal cortex; HO-2 expression was only selectively affected. In cerebellum, activity of NO synthase, but not that of HO, was reduced. Consistent with the possibility that carbon monoxide can generate cyclic GMP, the change in cyclic GMP level did not mirror the decrease in NO synthase. We suggest that glucocorticoid-mediated deficits in hippocampal functions may reflect their negative effect on messenger-generating systems.**

Acta Oncol 1995;34(3):329-34 **Relationship between tumour oxygenation, bioenergetic status and radiobiological hypoxia in an experimental model.** Nordsmark M, Grau C, Horsman MR, Jorgensen HS, Overgaard J. "Tumour oxygenation and bioenergetic status were measured in the same tumour and these results related to radiobiological hypoxia. A C3H mouse mammary carcinoma grown in the feet of CDF1 mice was used." "In conclusion, oxygen electrode measurements were sensitive to changes in tumour hypoxia whereas **the bioenergetic status alone seemed to be a less precise measure of hypoxia** in this tumour model. Furthermore, the present study demonstrated that tumour cells in vivo can actually maintain the bioenergetic status during a period of severe hypoxia."

Mutat Res 1998 May 25;400(1-2):439-46 **Mutagenesis induced by the tumor microenvironment.** Yuan J, Glazer PM **"Genomic instability is a commonly observed feature of tumors. Most investigations addressing the mechanism of tumor progression have focused on the genetic factors that may play a role. Growing evidence now suggests that, in addition to these endogenous factors, the exogenous environment within solid tumors may by itself be mutagenic and constitute a significant source of genetic instability. The tumor microenvironment is characterized by regions of fluctuating hypoxia, low pH, and nutrient** deprivation. Each of these microenvironmental factors has been shown to cause severe disturbance in cell metabolism and physiology. Both in vivo and in vitro data demonstrate that exposure of tumor cells to **adverse conditions can directly cause mutations, contributing to genetic instability. In this review, we will reexamine the current body of evidence on the role of the tumor microenvironment in inducing mutagenesis and consequent tumor progression."**

Lakartidningen 1995 May 24;92(21):2197-2198 **[Estrogen treatment caused acute attacks of porphyria].** [Article in Swedish] Wetterberg L, Olsson MB, Alm-Agvald I. Two female patients with acute intermittent porphyria, who received oestrogen skin pads as supplementary treatment for postmenopausal discomfort, developed severe psychiatric disorders with persistent confusion, aggression and paranoid reactions. Some decades earlier they had reacted with symptoms of acute porphyria following oral contraceptive usage. There is well documented evidence of the advisability of restrictiveness in the use of oestrogens in conjunction with acute porphyria, particularly in cases of patients with a history of hormone-related symptoms of acute porphyria. The putative mechanisms by means of which oestrogens may exert effects on neurotransmitters and peptides are discussed in the article. The authors would be grateful to hear from colleagues abroad who have treated patients with similar symptoms following postmenopausal treatment with oestrogens.

J Am Acad Dermatol 1994 Aug;31(2 Pt 2):390-392 **Porphyria cutanea tarda: pregnancy versus estrogen effect.** Urbanek RW, Cohen DJ Department of Medicine, Staten Island University Hospital, NY 10305. We describe the worsening of porphyria cutanea tarda in a young woman while she was taking oral contraceptives. However, she did not have an exacerbation during two pregnancies. We conclude that estrogens produced during pregnancy do not exert the same effect as orally administered medications that contain estrogen. The pronounced effect of oral ethinyl estradiol on the liver may be attributed to its first-pass effect on that organ.

Int J Radiat Oncol Biol Phys 1998 Nov 1;42(4):849-53 **The comparative effects of the NOS inhibitor, Nomega-nitro-L-arginine, and the haemoxygenase inhibitor, zinc protoporphyrin IX, on tumour blood flow.** Tozer GM, Prise VE, Motterlini R, Poole BA, Wilson J, Chaplin DJ.

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