α2μ-Globulin Nephropathy in White Ravens

In a recent article in *Scientific American*, Woodward and Goodstein (*1*) discussed the handling of data that do not support a given hypothesis. They illustrated their point as follows:

> an investigator might inductively infer, after observing a large number of black ravens, that all ravens are black. According to the inductive approach, good scientific practice consists in recording all that one observes and not just some selected part of it. The guiding ideal is to avoid any error that might slip in as a result of prejudice or preconception.

Later, the authors discussed the falsificationist ideas of Karl Popper in relation to the black ravens: "the observation of a single nonblack raven will falsify the hypothesis that all ravens are black."

Huff (*2*) recently discussed the role of cell proliferation in chemical carcinogenesis. At one point he discussed the ability of certain putatively nongenotoxic chemicals to induce renal cancer in male rats. The section in question is worth quoting in full.

> Meanwhile regulators and administrators should continue to be considerably cautious before embracing a purported mechanism—even a reasonable proposed hypothetical mechanism of carcinogenesis—as being pivotal to public health decisions. One example still garnering considerable debate centers on whether chemicals that induce cancers of the tubular cell epithelium of the kidney in male rats, concomitant with an increase in cell proliferation and testosterone-mediated α-2μ-globulin protein are relevant or useful to overall hazard identification for humans. The U.S. Environmental Protection Agency (for example) has embraced the concept that these experimental carcinogenic responses are not important to the human situation, whereas others (e.g., the Interational Agency for Research on Cancer) appear properly cautious, or have proposed equally reasonable alternative hypotheses. For example, and yet to be clarified, there are several chemicals known to induce this specific nephropathy syndrome that do not cause kidney cancers, and may or may not produce cancers in other organs; this lack of consistency must be explained.

Huff has seen some white ravens, but he has not identified them for other interested parties to study. I too have heard similar rumors, but I have yet to see a convening of the chemical structure, genetic toxicity, hyalin droplet data, and adequate rat cancer bioassay data for such a chemical. Given the importance of the issue under discusion, it would be valuable if Huff were to identify the chemicals to which he referred and present the relevant data for them in a focused paper. This would enable the commissioning of research to elucidate whether the α2μ-globulin male rat renal cancer hypothesis should be retained or abandoned. Equally, when the available data are scrutinized, and perhaps extended, it may be possible to retain the hypothesis for use under qualified conditions; for example, it may be concluded that the persistence or magnitude of the nephropathy, as opposed to its presence, is the critical parameter.

The surest way to make progress in assessment of the hazard posed to humans by rodent carcinogens is for exceptions to current models to be clearly identified and made the subject of active research. In the absence of a range of valid models, there will be only one: namely, that the rodent carcinogens dibenzanthracene and sodium saccharin present the same intrinsic carcinogenic hazard to humans.

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Response: α2μ-Globulin Nephropathy, Posed Mechanisms, and White Ravens

In our zest to discover the holy grail(s) of chemical carcinogenesis—read mechanism(s)—we seem to have short circuited the time-honed pattern of Koch's path of finding/proving scientific truth. As I understand the scientific paradigm of discovery, one must exhaust and counter all possible exceptions to the purported hypothesis before accepting the conjecture as truth. Further, if one exception to the rule persists—white ravens—then the hypothesis is either untrue or, at the most, must be modified to accommodate the uncertainty introduced by the exception. If several and varied exceptions exist, then the putative mechanism must be abandoned or be considered very limited in validity or application. Ashby (*1*) proposes that "it may be possible to retain the hypothesis for use under qualified conditions." Ideally, I have little difficulty with his suggestion; however, in practice, the workability of such nuances is nearly impossible. Recall that "as it happens often in science, a timid suggestion at the end of a discussion, a carefully worded hypothesis, become transformed in the next paper (often by other authors) into a fact" (*2*). Perhaps this is what happened to the α2μ-globulin hypothesis, without the attendant necessary search for white ravens. Some have associated this testosterone-mediated protein with tumors of the kidney in male rats. Importantly, a plausible rationale has yet to be offered to explain why certain α2μ-globulin-provoking chemicals fail to systematically induce renal tubular cell tumors. Early on, in fact, several of us have raised basic issues about this purported mechanism (*3–5*).

Hence, from my personal perspective in the sensitive area of human health and life, one must ignore the scientific urge to retain such tentative and "footnoted" hypotheses because proof, acceptance, and utilization for public health purposes must remain sacrosanct and on a firm foundation, especially when the use of said limited proof allows greater potential risks to be propagated or perpetrated. Nonetheless, the search for mechanisms of carcinogenesis—likely to be chemical specific—must continue.

In this vein, Ashby (*1*) urges me to identify the white ravens in the α2μ-globulin story. As that earlier paper, from which Ashby generously quotes my writing, was a more broadened view of the visible white ravens in the overall hypothesis of chemically induced cell proliferation and cancer (*6*), I did not identify the specific chemical exceptions to the α2μ-globulin hypothesis. I had given the names of those chemicals in previous papers (*7,8*) and did not think them particularly relevant in the cell proliferation paper quoted by Ashby. Further, I certainly believed that those professing the strength of the α2μ-globulin hypothesis would have been aware of these exceptions—aware of them and either explained the discrepancies or simply discounted them. In this communication, the chemicals that disobey the α2μ-globulin hypothesis are given.

I am pleased for the opportunity to list these chemicals, together with brief explanatory passages for each. So far, there are at least four chemicals that instigate the α2μ-globulin-syndrome yet do not induce tumors of the kidney in male rats: gabapentin, an antiepileptic drug; lindane, an insecticide; decalin, a solvent; and trimethylpentane, the prototypical α2μ-globulin inducer present in gasoline. Additionally, another chemical induces the α2μ-globulin sequela but causes two different cellular types of tumors of the kidney (dimethyl methylphosphonate). One

chemical has similar α2μ-globulin responses in both control and exposed animals (*t*-butyl alcohol); another chemical induces α2μ-globulin, yet causes the same renal tubular cell tumors in both sexes (hexachlorobenzene).

Importantly, two additional chemicals presumed to involve α2μ-kidney tumors (and thus are regarded by some as irrelevant to humans) have now been shown to be associated with kidney cancers in humans: gasoline (*9,10*) and trichloroethylene (TCE) (*11,12*). Actually, TCE, one of several chlorinated hydrocarbon solvents once predicted and thought to activate this syndrome, has not been shown to induce α2μ-globulin, but is interesting to mention simply for historical completeness. Another solvent linked to human cancers, perchloroethylene, causes renal tubular cell tumors at concentrations that neither increase protein droplets nor accumulate α2μ-globulin in proximal tubules of male rats (*4*). If readers are aware of other chemical exceptions, these should be documented and reported as well.

Gabapentin. In long-term feeding experiments, gabapentin caused adenomas and adenocarcinomas of the pancreas in male rats (*13,14*). Also, this chemical has been reported to induce α2μ-globulin; yet, the predicted renal tubular cell tumors were not found in these studies (*15*). Maintenance antiepileptic doses for this therapeutic agent range from 1,200 to 2,400 mg/day, but "the dose required to achieve control of seizures may be much higher" (*16*). Thus, doses of gabapentin may be as high as 50–100 mg/kg in humans, not far removed from the lowest dose causing tumors in animals.

Lindane. The γ-isomer of hexachlorocyclohexane, lindane, induces α2μ-globulin nephropathy (*17*) without causing tumors of the kidney in long-term carcinogenicity studies (*18*). Tumors of the liver in mice and of the thyroid gland in rats were induced following long-term exposure to lindane (*18–20*).

Decalin. Decalin is probably the first chemical for which no empirical connection could be made between induced hyaline droplet-α2μ-globulin nephropathy syndrome and cancer of the renal tubular epithelial cells (*21*). In these experiments, male and female Fischer 344 rats and female C57Bl/6 mice were exposed to decalin vapor at concentrations of 0, 5, or 50 ppm for 13 weeks, followed by a 19-month observation period. After 13 weeks of exposure to decalin, male rats exhibited the classical α2μ-globulin nephropathy, characterized by hyaline droplets, necrosis, and intratubular casts. At the end of the post-exposure period, male rats exhibited dose-related chronic progressive necrosis with accentuated tubular degeneration, medullary mineralization, and papillary hyperplasia; however, no tumor of the kidney was reported for male rats or for any other sex–species–exposure group.

Whether this exposure regimen was rigorous enough to make decalin the first α2μ-globulin-associated nonkidney carcinogen remains to be confirmed. Yet, until ongoing experiments by the National Toxicology Program using both Fischer and NBR rats are completed and reported, this conclusion would appear reasonable, given that the α2μ-globulin-syndrome was extant and chronic nephropathy was dose related at the end of the 22-month experiments. Moreover, as evidence of a carcinogenesis experiment, incidences of tumors of the pituitary gland were increased in male rats and in female mice.

Trimethylpentane. Isooctane (2,2,4-trimethylpentane; TMP) is a major component in gasoline and the putative α2μ-globulin inducer thought to impart the observed carcinogenicity to gasoline (*22*). Activity resides apparently in the TMPOH metabolite (2,4,4-trimethyl-2-pentanol). What is interesting in this scenario is that TMP, the prototypical α2μ-globulin inducer, had not been tested for carcinogenicity during the time much of the mechanistic effort was going on. Thus, one did not know if TMP would in fact be the agent responsible for the gasoline-induced kidney toxicity and carcinogenicity in male rats or liver tumors in mice (*7*).

Now this chemical has been evaluated by the Instituto Ramazzini for carcinogenicity in a long-term bioassay, and the finding is that trimethylpentane is not carcinogenic for the kidney or for any other organ in male or female Sprague Dawley rats exposed to this chemical for their life time (F. Belpoggi, personal communication). And the observed nephropathy was typical of what is seen in these aged rats. Thus, not only do these negative findings make one further question the canon of α2μ-globulin inducers per se in the carcinogenic process, but also places full doubt on the identity of the causative agent in gasoline responsible for tumors of the kidney and liver.

Dimethyl methylphosphonate. Dimethyl methylphosphonate (DMMP), used as a flame retardant and as a nerve gas simulant, was given by gavage for 2 years to F344 rats and to B6C3F$_1$ mice (*23,24*). These authors concluded that

> The spectrum of kidney lesions seen in the male rat given DMMP is similar to that seen after the long-term administration of a variety of other chemicals including unleaded gasoline, hydrocarbon solvents, and 1,4-dichlorobenzene.

The authors go on to suggest that the kidney lesions were associated with hyaline droplet nephropathy and, although not measured, specifically to α2μ-globulin.

Two observations tend to complicate this notion: first, DMMP induced hyperplasia of the tubular and of the transitional cell epithelium (the latter not ordinarily associated with α2μ-globulin), and second, DMMP caused not only tubular cell adenocarcinomas but also transitional cell papillomas and carcinomas (the latter tumors not ordinarily associated with α2μ-globulin). Logically then, because this combination of lesions and kidney tumors have never occurred in tandem with respect to α2μ-globulin, one might suppose that dual mechanisms are at play. Or one might parlay that DMMP alone caused these lesions and the co-occurrence of α2μ-globulin is mechanistically irrelevant, simply being a coincidental biomarker of exposure or a carrier molecule as some have proposed (*3-5*). In any event, more effort needs to be directed towards understanding the mechanism(s) underlying this duality phenomenon.

t-Butyl Alcohol. *t*-Butanol (TBA), used in making perfumes and cosmetics, causes increased formation of hyaline droplets in the kidneys of male rats (*25,26*). TBA did induce kidney tumors, yet the coincidental occurrence of other lesions in both controls and exposed animals makes one pause in declaring this association as causal. Interestingly, as in DMMP, TBA induces hyperplasia of transitional epithelial cells in addition to the typical α2μ-globulin nephropathy. Also, TBA causes tumors of the thyroid gland in mice, another new finding for α2μ-globulin chemicals. Suspicion about this posed mechanism is also raised by the observations of similar toxicity in both controls and exposed male rats: mineralization, severity of nephropathy, and renal tubular cell hyperplasia. The obvious questions are why the controls exhibit toxicities of the kidney very similar to the exposed animals although they were not exposed to TBA or why the exposed animals only had lesions similar in severity and incidence to the controls. This could mean no more than a typical late stage nephropathy seen in most male rats, and in these studies, female rats also exhibited end-stage nephropathy.

Using step-sectioning of the kidney, tubular cell tumors were found in 16% of controls versus 25%, 38%, and 26% of the three TBA-dosed groups of animals. The only apparent difference between controls

and treated male rats was the occurrence of hyaline droplets, which did not induce any concomitant differences in toxicity and only a marginal difference in tumor incidence. In fact, standard sectioning revealed one renal tumor in controls versus three, four, and three in TBA animals, not an overwhelming response, especially if one looks at the results to confirm the role of α2μ-globulin in the carcinogenesis process.

Hexachlorobenzene. Hexachlorobenzene (HCB) is an example of a chemical that induces the α2μ-globulin nephropathy syndrome in male and female Sprague Dawley rats (*27*) and causes cancer in several species [rat, liver and kidney (*28*); hamster, liver and thyroid gland (*29*); mouse, liver (*29*)] and in a two-generation study in rats, parathyroid and adrenal glands (*20,30*). HCB is the first chemical identified that exhibits the α2μ-globulin nephropathy sequelae, and induces renal cell tumors in both genders of rats.

Tubular cell tumors of the kidney were found more often and apparently earlier in male rats than in females (*28*). In these HCB studies, even lower exposure regimens would have likewise been associated with renal tumor induction, especially in males. In females, one might view the dose response–tumor induction pattern as being perfectly correlated with chemical exposure (that is, double the exposure concentration and double the tumor response) although tumor rates were not age-adjusted for possible differences in survival (*28*). Further, three types of the liver tumors were observed in HCB-exposed Sprague Dawley rats, with females being more responsive than males. Whether competing risk factors or the commonly later-occurring kidney tumors in females influenced these numerical differences of HCB-induced renal carcinogenesis remains to be ascertained.

Using two protocols (100 mg/kg/day for 15 days and 50 mg/ kg/day for 50 days), Bouthillier et al. (*27*) observed only in males degenerative and regenerative cellular foci, along with accumulation of protein droplets, in epithelial cells of the proximal tubules in both HCB experiments. Renal functional alterations were seen with the longer exposure. α2μ-Globulin was increased 11-fold in male rats compared to controls (apparently not measured in females), and HCB was found to be bound reversibly to α2μ-globulin. Authors conclude "that HCB induces a male rat specific nephropathy that could explain the higher incidence of kidney tumors in male rats compared to female rats," and "protein droplets . . . are considered to represent the initial step toward specific kidney tumor formation in male rats compared to female rats" (*27*).

Because HCB induces tumors of the kidney in female as well as in male rats (by different mechanisms? and do these different mechanisms influence renal tumor induction and incidence in male rats, too?) and HCB induces various and unusual tumor types at high incidences in both sexes of three rodent species, other interpretations must be considered. Alternate theories have been proposed (*3–5*) and more research needs to be done before one continues to discount chemical-induced tumors in the male rat kidney as being irrelevant for assessing risks posed to humans.

For example, Fowler and DuVal (*30*) have identified a specific cleavage product of α2μ-globulin as the high affinity lead-binding protein responsible for deposition of lead in the kidney of male rats. A similarly functioning protein has been demonstrated in human kidney (*31*). This human lead-binding protein has mass and charge similar to that of the α2μ-globulin detected in rat kidney; however, the two proteins apparently differ in immunological reactivity. Thus, chemicals (or metabolites) that induce α2μ-globulin nephropathy and kidney cancer in male rats may logically cause kidney toxicity and/or carcinogenesis in humans after reacting with the lead-binding protein or by another mechanism. Of course, this assumes one accepts both purported mechanisms.

Of the chemicals observed to induce hyaline droplets in NTP long-term studies, only *d*-limonene caused the singular occurrence of tubular cell neoplasms without also inducing other neoplastic responses in male or female rats or mice. Therefore, only one of the chemicals we have studied that induce hyaline droplets is exclusively a male rat kidney carcinogen (*3,32*). This is important in understanding the mechanistic pathways for the carcinogenic responses associated with these chemicals and permits the likelihood that a chemical may have multiple mechanisms, or alternatively, it may indicate that binding to α2μ-globulin has little mechanistic function other than to locate and retain the chemical or metabolite in the kidney. Further, the great majority of chemicals that exhibit a gender difference in the induction of kidney cancers has not been shown to increase α2μ-globulin accumulation in the kidney; therefore, the bases for the higher prevalence of tumors of the kidney in male rats has not been established. Likewise in humans, kidney tumors develop more frequently in males than in females (*3,7*).

Short (*33*), for instance, citing his work and others, suggests that

> similar correlative studies with a number of other renal carcinogens and noncarcinogens are warranted before general conclusions can be made. Cell proliferation is excessively elevated in tubules affected by chronic progressive nephropathy, but the significance of the lesion to renal carcinogenesis is unclear. Elucidating mechanisms of renal cell proliferation are necessary for our understanding of cause and effect relationships.

And cell proliferation is only a part of the multistage process of carcinogenesis (*6,34*).

In 1969, Foulds (*35*) provided us with an insightful cautionary note that may be relevant today in our quest for defining mechanisms of carcinogenesis:

> One of the greatest evils in modern cancer research is the abuse of definitions that are based on the preconceived ideas of individual investigators highly expert over an extremely narrow range of neoplastic phenomena . . . ; they presume as axiomatic what remains to be proved or, sometimes, what has already been disproved.

Given the above exceptions to the rule, perhaps a new look needs to be given to the postulated association between α2μ-globulin production and renal tumors in male rats and their relevance to public health. After all, 1) the accumulated data given above preclude any blanket endorsement of the α2μ-globulin-mechanism, especially given that 2) so far, only one chemical, *d*-limonene, has been associated singularly with the α2μ-globulin syndrome and kidney tumors in male rats, with no other tumors in female rats or in male or female mice; 3) in all cases that have been proposed to evoke the α2μ-globulin theory, none of the chemicals exhibit any greater incidences of renal cell tumors than do non-α2μ-globulin inducing chemicals, and most exhibit poor dose response curves; and 4) the majority of chemicals that preferentially cause tubular cell tumors of the kidney predominantly in male rats do not provoke α2μ-globulin, thus, not explaining the significant gender differences in this and other chemically induced specific organ site tumors (and in humans as well). Regarding this last point, i am convinced that if bioassays were conducted for longer periods of time, e.g., 30–36 months, gender discrepancies for tumors of the kidney would become moot.

In our rightful quests to identify and define mechanisms of chemical carcinogenesis, we must remain vigilant during our pursuit to overcome any tendency to endorse or accept a mechanism before incontrovertible proof exists. For instance, early in the α2μ-globulin metaphor, some were proposing that chemicals shown to induce α2μ-globulin in short-term studies

need not be tested in long-term bioassays because the results were predictable. This has been disproved.

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PBBs: Potential Effects in Children

Colborn et al. (*1*) describe thyroid hormone deficiency in Great Lakes salmon and state that research has ruled out iodine deficiency as the cause. Great Lakes herring gulls also have enlarged thyroid glands; it has been suggested that chemicals in the lakes can block thyroid hormones, but a specific chemical has not been identified. I would like to call attention to the article by Bahn et al. (*2*) in which the authors describe cases of primary hypothyroidism in men employed in the manufacture of polybrominated biphenyls (PBBs). The observation of thyroid abnormalities in animals exposed to PBBs and PBB oxides supports the hypothesis that exposure to PBBs or other brominated compounds is causally related to the hypothyroidism in these men. Perhaps the thyroid deficiencies observed in Great Lakes salmon and herring gulls are a result of the large-scale contamination of the Michigan environment with PBBs in the early 1970s. Furthermore, dairy cattle were thought to be suffering from an ailment similar to iodine toxicity early in the PBB contamination episode (*3*). Perhaps the halogenated chemicals have similar actions. It has been suggested that the thyroid hormone deficiency may result from the similarity in structure of thyroid hormone and the biphenyl compounds; thus,