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IMMUNOSTIMULATION OF CANCER *VERSUS* IMMUNOSURVEILLANCE

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Summary A moderate antitumor immune reaction is optimal for tumor growth and most (perhaps all) untransplanted tumors, rather than being inhibited, are probably dependent, at least early in their progression, upon the immune reaction. Some tumors, as for example most human skin tumors, have a higher incidence in immunodepressed patients than they do in the general population. This could mean that the normal immune reaction usually inhibits the growth of these tumors. More probably, the increased incidence in immuno-depressed heart and kidney transplant patients is caused by a lowering of a tumor-stimulatory immune reaction to a level that is even more stimulatory. Other tumors, such as human mammary tumors, have, in contrast to the skin tumors, a lower than expected incidence in immunodepressed patients. Mammary tumors are postulated to possess, on average, a low immunogenicity that arouses an immune reaction that is usually equal to or less than that required for optimal tumor growth; a further lowering of the reaction, as occurs in heart or kidney transplant patients, thus results in a lowered tumor incidence. In such patients, an immunostimulating adjuvant or vaccine might, we predict, sometimes accelerate rather than inhibit tumor growth.

That tumor-specific immune reactions can inhibit the growth of at least some types of cancer implants has been demonstrated beyond a reasonable doubt[1]. In fact, it was the reluctant acceptance, in the late '50s, of this observation that led to the idea of immunological surveillance[2]. According to this theory, most tumors would be eliminated at their inception by an immune mechanism that had evolved in higher organisms for this specific purpose. Such an appealing idea[3]! However, there were certain inconsistancies. The major inconsistency was that immunodepressed patients, although developing a large increase in the incidence of skin cancers and lymphomas, did not develop an excess of major killers such as breast, colon and lung cancers[4]. In fact, most types of cancer showed little or no increased incidence in immunodepressed patients nor was there an increase, again with the notable exception of lymphomas, in immunodeficient athymic nude mice[5].

The second blow to the surveillance concept, albeit perhaps not as devastating, was the fact that implants of so-called «spontaneous tumors» in rodents usually failed, in striking contrast to most of the tumors induced with chemical carcinogens or viral agents, to be inhibited in their growth by a previous immunization[1]. If these tumors were relatively or even completely nonimmunogenic, how could immunological surveillance inhibit their occurrence? The usual answer was that spontaneous tumors were probably a small subpopulation of the universe of tumors and that this subpopulation, by virtue of nonimmunogenicity, escaped surveillance. The apparent nonimmunogenicity of spontaneous tumors was interpreted as showing that immunoselection, and therefore immunosurveillance, was alive and well. Unfortunately for the surveillance theory, several studies from our own laboratory showed that immunogenicity was a direct result of carcinogen-ac-

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tion; tumors that arose in the immunologically deficient confines of diffusion chambers or in tissue culture were detectably immunogenic only when they were induced by a chemical carcinogen and in some cases not even then[6-8]. Immunological surveillance was apparently not a very significant factor in determining the presence or the absence of tumor-immunogenicity; non-immunogenic tumors were not a result of immuno-selection.

Despite these observations, the next decades saw the publishing of a vast literature in which the effects of immunodepression or of immunoenhancing procedures on the incidences of tumors in various experimental tumor systems were generally interpreted within the framework of the surveillance hypothesis, often on the most nebulous evidence. In 1975, Stutman published an extensive review of this literature and came to the conclusion that, in aggregate, manipulation of the immune capacity usually had little or no effect on tumor incidence and he suggested that, with the exception of some virally-induced tumors, the immune response did little either to inhibit or to promote oncogenesis[9]. Many studies seemed to support the surveillance hypothesis, but just as many did not. Nonetheless, immunosurveillance remains central to the thinking of many investigators and most clinicians.

In the meantime, unknowingly following the lead of Spärck[10], one of us had begun to think that immunity might often stimulate rather than inhibit tumor development[11, 12] and in the subsequent two decades evidence supporting this idea has steadily accumulated. It is our purpose in this paper to present this evidence in a somewhat new and hopefully persuasive way, concentrating largely on the work with which we are most familiar, that of our own laboratory. Other aspects of this field have been extensively reviewed[13-16].

Immunostimulation of transplanted tumors

It was probably the observation that genetic disparity between a mother and her fetus might favor the growth and well-being of the fetus that, among a variety of other types of suggestive observations, led one of us to explore the possibility that immunity might sometimes stimulate rather than inhibit tumor growth[11, 16]. The hypothesis, as originally formulated, suggested that a quantitatively weak immune reaction might stimulate tumor growth whereas a quantitatively stronger reaction of the same type might be inhibitory[11]. If the hypothesis were correct, the early immune reaction in response to an incipient tumor would necessarily be stimulatory and there would be little room for the concept of immunosurveillance.

The stimulation hypothesis received support from the observation that the titration of specifically immune spleen cells against tumor cells by inoculating mixtures of the two subcutaneously into immunodepressed mice (Winn tests) gave a biphasic growth curve; as predicted, low proportions of immune cells stimulated, but larger proportions inhibited tumors growth relative to the effect of non-immune spleen cells[17]. Subsequently, a large number of studies in our own and in other laboratories have given essentially the same result by a variety of means and have extended the observations to show that a variety of immune effectors, *i.e.*, immune macrophages, immune lymphocytes, NK cells, and specific immune antiserum could all stimulate tumor growth when present in low proportion; perhaps this is a general property of all the various effectors of the immune reaction[18].

The mechanisms that underlie the biphasic curve remain a matter for speculation. Some data suggest that stimulation and inhibition may sometimes be mediated by distinctly different cell populations[19, 20]; the immune mechanism is exceedingly complex and it is quite likely that both quantitative and qualitative aspects of the immune response combine to determine the effective magnitude of the reaction and whether stimulation or inhibition of the tumor occurs in any given instance. The work with immune serum suggests a basic quantitative mechanism. Perhaps the degree of cross-linking of surface receptors on the tumor cell is a major determiner of the outcome.

Not only does the immune reaction regulate tumor growth; there is some evidence that it may also regulate biological progression. Hammond has published data suggesting that the speed and extent of biological progression (*i.e.*, change to a more malignant phenotype) among chemically induced bronchial carcinomas of the hamster, both among serially transplanted tumors as well as among tumors followed in the primary hosts after repeated partial excisions, were directly re-

lated to the experimentally varied immunological capacities of the hosts; the *greater* the probable immune reaction, the *faster* was the progression[21]. Conversely, Hammond's data compliment other data that showed that in some 25% of cases, tumors that were passaged in immunologically deficient nude mice became *more* differentiated and presumably less malignant than were the same tumors in the hosts of origin[22, 23].

Immunostimulation of untransplanted primary tumors

The immunostimulation or immunoinhibition of transplanted tumors does not necessarily predict what the effect of immunity may be on tumor induction or on the growths of untransplanted tumors growing in their primary hosts. Can the same biphasic reaction be demonstrated? Experiments to this end are more difficult and time consuming than are studies with transplanted tumors or with tumors growing *in vitro*.

An important point to be noted is that the growth of a primary *de novo* tumor does not maximally immunize its host, a point that the very existence of the tumor may suggest. This point is further shown by the observation that the primary host, after removal of the tumor, does not usually resist the growth of a subsequent implant of that same tumor and that the primary host is, in comparison with secondary syngeneic hosts, difficult to immunize[24]. In fact, challenge of the primary host with an implant of the same tumor sometimes results in *better* growth of the challenge implant than is observed among control implants made to naive syngeneic recipients, a result that is not dependent upon the transient immunosuppressive action of the carcinogen[25-26]. Apparently the growth of the tumor from a very small nidus, in either primary or secondary hosts, leads to a relative lack of effective immunity, perhaps because of immunological tolerance and/or of enhancement by blocking antibodies and/or of the calling forth of suppressor types of immune cells and/or of other mechanisms[18]. In secondary hosts this phenomenon has been called «sneaking through»[27-30]. The relative failure of the untransplanted tumor to produce a tumor-inhibiting immunity in the primary host despite an often easily demonstrable immunogenicity in syngeneic secondary hosts would seem to open the door to the possibility of therapy and/or prevention by immune manipulation.

Despite the resistance of the primary host to immunization against tumor implants, immunity does modulate tumor incidence. There are essentially two different approaches to discovering the role of the immune reaction in the primary host: one is to vary the immune capacities of the tumor hosts or, alternatively, to vary the innate immunogenicities of the tumors.

One of our approaches to the problem made use of the observation in our laboratory that when sarcomas were induced by the subcutaneous placement of methylcholanthrene-containing paraffin wafers, there was, on average, a direct correlation between the carcinogen concentration in the wafers and the immunogenicities of the resulting tumors that was largely independent of immunoselection[31, 32]. It had also been observed that there were marked differences in susceptibility to methylcholanthrene-induced sarcomagenesis among various inbred strains of mice. Taking advantage of these observations, L. Prehn and E. Lawler found that those mouse strains that were relatively most susceptible to wafers with a high concentration of carcinogen were the mouse strains least susceptible to wafers with a low carcinogen-concentration and vice versa[33]. Furthermore, with either dosage of carcinogen, the mouse strain most susceptible to methylcholanthrene was made *more resistant* to oncogenesis by radiation-induced immunodepression, but the least susceptible strain was made more susceptible[34]. Apparently, reducing the immune reaction, either by a reduced dosage of carcinogen or by whole-body radiation, aided the growth of tumors whenever the immune reaction would otherwise have been large, but interfered with the development of tumors whenever the reaction would have been small. Thus, it is evident from these results that an *intermediate* level of antitumor immune reactivity was optimal for tumor production; in this system, either a greater or a lesser immune reaction produced fewer tumors.

Bernfeld and Homburger did not confirm the above results; no alteration in the relative susceptibilities of strains was produced by altering the dosage of carcinogen. However, these investigators delivered the carcinogen in oil rather than in a solid paraffin wafer[35]. A relationship between

tumor immunogenicity and the *dosage* of carcinogen was not seen by Stutman who also delivered the carcinogen in oil[9]. The reasons for this have not been explored, but we suggest that the immunogenicities of tumors are probably related to the *concentration* of carcinogen rather than the dosage; the concentration of the carcinogen at the surface of the target cell, when delivered in oil, might remain much the same regardless of the amount of oil delivered. Thus the concentrations of carcinogen in the experiment by Bernfeld and Homburger may not have been varied and there may have been, therefore, no systematic alteration of tumor immunogenicities.

Our former college, Dr. H.C. Outzen, destroyed the immune capacities of mice to varying extents by giving varied dosages of X-irradiation[36]. He then transplanted to them skin grafts that had been exposed to a moderate concentration of methylcholanthrene; the transplantation served as a promoter. Papillomas appeared in the skin grafts in a biphasic way, *i.e.*, tumors appeared earlier and most numerously in the animals whose immune systems had received an *intermediate* dosage of radiation and that were shown to have a modest residual immune capacity. In this experiment, interpretation was simplified by the fact that the tumors arose in skin that had not been irradiated and in animals that had not received carcinogen. A similar biphasic effect on carcinogenesis was achieved by normalizing to varying degrees the immunological capacities of nude mice by injecting varying numbers of normal spleen or thymus cells[37]. Thus, a modest level of immune capacity was apparently optimal for oncogenesis. Interestingly, in basically similar experiments by Marc Lappé, in which the concentration of methylcholanthrene was approximately doubled, a biphasic curve was *not* observed[38]. We suggest, as the most probable explanation, that in Lappé's experiment, because of the probably very high average immunogenicity of the tumors that resulted from the doubled carcinogen concentration, only one end of the biphasic curve was explored.

A different approach using the skin carcinogenesis system was reported from another laboratory[39]. The investigators confirmed that autoantibody to skin could be induced in mice by injections of skin; syngeneic injections produced low levels, but xenogeneic injections produced very high levels. It was then shown that syngeneic injections promoted the appearance of papillomas in carcinogen-initiated skin, but that xenogeneic injections failed to promote. Again, a low but positive level of immune response, in this case antibody, correlated with the greatest tumor production.

In a somewhat similar experiment, the immune capacities of mice, thymectomized as adults, were varied by R. Prehn by exposing them to a maximally tolerated dosage of x-irradiation and then restoring the immune capacities to varying degrees by giving the mice varied numbers of normal spleen cells[40]. Sarcomas were then induced by subcutaneously-placed wafers of Millipore filter impregnated with embedding wax that contained methylcholanthrene. In response to moderate concentrations of the methylcholanthrene, animals with *intermediate* levels of immune restoration were most susceptible; greater or lesser levels of restoration produced fewer tumors. Once again, a low but positive level of immune capacity was apparently optimal for tumor production. However, animals exposed to very low concentrations of carcinogen did not show any detectable influence of immunomodulation on tumor production suggesting the possibility that the tumors may have possessed, perhaps in common with many «spontaneous» tumors, too little immunogenicity to register on the immune system.

Newborn thymectomy interferes with the development of the immune system of the mouse and titration of the quantity of thymus (by thymectomy and by making thymus grafts) against the growth of a transplanted mammary tumor produced a beautiful biphasic curve; thymectomy reduced tumor growth, addition of 3 extra glands stimulated growth, but 5 extra glands reduced tumor growth below that seen in intact control mice[41]. Also, thymectomy at the third day of life, but not before the second day or after the fifth, results in a variety of organ-specific autoimmune reactions[42]. The autoimmune lesions were often hyperplastic and, in the case of the ovary, sometimes became neoplastic[43]. In the case of autoimmune gastritis, L. Prehn was able to transmit the disease with spleen cells from 3-day thymectomized donors before the donor's gastritis had occurred[44].

The literature concerning the effects of thymectomy on chemical oncogenesis is confus-

ing, but some of the reasons for the varied results are now apparent. L. Prehn showed that the effect of 3-day thymectomy upon subsequent carcinogenesis was critically dependent upon the concentration of carcinogen and thus, probably, upon the immunogenicities of the tumors; 3-day thymectomy reduced the oncogenic effect of low concentrations of carcinogen, but increased the oncogenic effect of high[45]. Thus, once again, it appears that the immune capacity of the host interacted with the intrinsic immunogenicity of the tumor and that an *intermediate* resultant level of immune reactivity was optimal for tumor production.

The importance of the immunogenicity of the tumors in determining the effect on tumor development by thymectomy of the new-born is also illustrated by work from other laboratories. Mammary tumors induced by the mouse mammary tumor virus (MTV) have very little immunogenicity when tested in virus-infected animals, but those induced by a hydrocarbon carcinogen tend to be strongly immunogenic. Martinez[46], as well as others[9], reported that early thymectomy *lowered* the incidence of virus-induced mammary tumors, but Johnson reported that new-born thymectomy *accelerated* the appearance of the chemically induced[47]. Although the two sides of this work were done by different authors at different times and under somewhat different conditions, it again appears that interfering with immune capability increased the rate of occurrence of the highly immunogenic tumors, but lowered the incidence of the tumors of lesser immunogenicity, suggesting once more that an *intermediate* level of immune reactivity against the tumor is optimal for tumor development.

D. Murasko and R. Prehn investigated the effects of varying the immunizing dosage of inactivated Moloney murine leukemia virus (M-MuLV) on the induction of tumors by a subsequently inoculated standard dosage of Moloney sarcoma virus (M-MuSV)[48]. Mice immunized with high viral dosages developed significantly fewer tumors than did non-immunized control mice, but those immunized with low dosages showed a significantly *increased* tumor incidence. The enhanced growth could be abolished by irradiation of the mice with 450 rads 24 hours prior to challenge. Thus, the biphasic curve is apparently not confined to tumor production by oncogenic hydrocarbons.

G. Bartlett and R. Prehn each independently performed almost identical experiments and with almost identical results despite the use of different mouse strains; the combined data involved 154 tumors, each induced by the same concentration of methycholanthrene[3]. Each tumor was assayed for its immunogenicity in syngeneic secondary hosts by the classical implantation-excision-challenge type of test in 15 to 20 test animals and an equal number of unimmunized controls. A wide spectrum of immunogenicities was found. The immunogenicities clustered around two distinct levels, one high and one low. It seems probable, albeit unproven, that, in accord with the ideas of Coggin[49], the lower cluster may have represented the immunogenicity of oncofetal antigens while the higher may have represented immunogenicity due to those antigens plus carcinogen-induced, individually tumor-specific antigens. The striking feature was that the tumors of *shortest* latency were characterized by immunogenicities that were *intermediate* between these two levels. That the latency was determined by the immune reaction was shown by the fact that tumors initiated in the absence of an immune reaction exhibited a similar pattern of immunogenicity levels, but there was no correlation with latency. This experiment showed that there was an optimum level of immune reaction for tumor production that was of *intermediate* strength and that the untransplanted tumor in the primary host did not usually adjust its immunogenicity to produce that optimal level. Although immunoselection has been demonstrated among carcinogen-induced tumors upon repeated passage[50, 51], selection apparently had little effect on the immunogenicities of tumors in the primary host and most tumors retained immunogenicities that were distinctly higher or lower than that which was optimal for tumor arisal.

Implications

The experiments we have discussed all point to the same conclusion; there is a level of immune reaction to tissue antigens, greater than nil, that is optimal for tumor development and growth. The biphasic effect of immunity on growth is probably quite general; there are many examples of hyperplasia associated with the lymphoid activity

produced by weak autoantigens[11, 18]. Even normal histocompatibility antigens seem to behave like the tumor antigens; for example, skin transplantation across a minor histocompatibility barrier may produce hyperplasia rather than rejection of the graft[52]. Thus, the growth of tumors possessing a low average immunogenicity, *i.e.*, most tumors induced with a low oncogenic stimulus or arising spontaneously, would, in all probability, almost always be stimulated by any immune reaction they might manage to arouse or that a supposedly therapeutic vaccine might produce.

The fact that the immune reaction to a particular type of primary tumor is sometimes greater and sometimes less than is optimal for tumor growth explains, we believe, why Stutman, in his exhaustive review, concluded that immunity probably has little effect, either inhibitory or stimulatory, on the incidence of primary tumors.[9]

The mouse model suggests that whenever a much higher tumor incidence is produced by immunodepression, as occurs in skin tumors in kidney and heart transplant patients, one can conclude that, in such cases, the immune reaction is of much greater magnitude than that which would maximally stimulate growth. Reduction of this large reaction to a more stimulatory level in the immuno-depressed patients would, therefore, favor tumor growth. Conversely, vaccines or immunity enhancing adjuvants would be expected to inhibit the growths of such tumors or at least reduce the degree of growth stimulation. It should be noted that the high incidence of leukemias and lymphomas in immunodepressed patients is not interpretable; these are tumors that arise from elements of the injured immune system *per se* and their high incidence may therefore have causes unrelated to the growth stimulating or growth retarding effects of any immune reaction.

Colon cancer may illustrate the therapeutic potential of vaccines or adjuvants on tumors which have a higher than expected incidence in immunodepressed patients. The incidence of this tumor, in marked contrast to rectal cancer, is apparently higher in immuno-depressed patients than it is in immuno-competent subjects (Stewart, T; personal communication-1996). Aspirin administration induces the expression of HLA-DR in colon cancer cells[53] and HLA expression has been correlated with immune inhibition of cancer growth[53]. It thus appears that aspirin administration, which markedly reduces the risk of colon cancer, may act as an adjuvant to increase the immune reaction and thereby lessen the degree of immunostimulation and perhaps to even produce an absolute inhibition of the growth of these cancers.

The work in the mouse further suggests that there are probably tumor types that, in most patients, induce an immune reaction only somewhat greater than that required for maximal tumor stimulation; in this case, immunodepression might lower the reaction to a level somewhat lower than that which would optimally stimulate the growths of these tumors and thus the observable effect on tumor incidence might be close to nil. Thus, the incidences of some tumor types, even if the tumors are immunogenic, might show little or no change in heart and kidney transplant patients, a prediction that may conform to observation[54]. However, raising, rather than lowering, the level of antitumor immunoreactivity among such tumors would be expected to reduce the stimulatory level of the reaction to a less stimulatory or even inhibitory level and thus might often be therapeutic; such may be the mechanism of the effect of BCG on superficial bladder cancers[55].

The mouse model further predicts that still other tumor types might usually produce a less than growth-maximizing level of immunogenicity and might therefore exhibit a lower than anticipated incidence in immunodepressed patients. The data now available seem to conform to this expectation, especially since the discovery by Stewart *et al* of the unexpectedly low incidence of mammary tumors in heart and in kidney transplant patients[54]. The mouse model suggests that the majority of human mammary tumors (or other tumors that may have a lowered incidence in transplant patients) probably induces an immune response whose magnitude, in immuno-competent subjects, is either close to or *less* than that which would maximally stimulate tumor growth; therefore, a reduction of the immune reaction to such tumors results in a decreased immunostimulation of tumor growth and a lowered tumor incidence. Conversely, increasing the immune reaction in such patients by vaccination or other means might sometimes produce accelerated tumor growth especially if the immunogenicity of the tumor were quite low.

Tumors at an early stage of their biological progression are probably more dependent upon the immune reaction and may therefore be more immunogenic than are tumors at later stages of their evolution. During progression, the dependency upon the immune reaction probably lessens just as the dependency of a hormonally dependent cancer lessens with time[12]. A larger lymphoid reaction, that may be associated with a better prognosis, may simply be a marker of a tumor that is at an earlier stage of its biological progression and for that reason has a better prognosis. Such a mechanism seems particularly obvious in skin lesions; early lesions of both squamous cell carcinoma and malignant melanoma are characterized by relatively heavy lymphoid infiltrates which characteristically become less prominent as the lesion progresses[56].

Conclusions

The probable fact that the growths of many and perhaps all tumors are stimulated, at least initially, by immune reactions does not imply that immunoprevention and/or immunotherapy cannot eventually be successful. It does imply, we believe, that blindly raising the level of an antitumor immune reaction without knowing whether the reaction, *in that patient,* is less than or greater than is optimal for the growth of *that particular tumor* might do harm. It cannot be assumed that a greater immune reaction will always be beneficial to the patient; it may not be incidental that most successful current therapies probably interfere with, rather than increase, any antitumor immune reaction that may be present.

Despite these problems, the observations thay many and perhaps all tumors may be immunogenic, that the immunity they arouse is less than the maximum that experimental manipulations can produce, and that artificially-induced immune reactions of large magnitude may, in many cases, be less stimulatory to tumor growth and might, in some circumstances, be absolutely inhibitory, suggests to us that immunotherapies will ultimately be successful. There is a difficult path ahead, but the tumor immunologist can, with justification, be optimistic.

Resumen

Inmunoestimulación versus *inmunovigilancia en cáncer*

Una respuesta inmune moderada es óptima para el crecimiento tumoral y la mayoría (tal vez todos) los tumores no transplantados serían dependientes de la respuesta inmune, por lo menos al inicio de su desarrollo. Algunos tumores, como por ejemplo la mayoría de los tumores de piel, son más frecuentes en pacientes inmunodeprimidos que en la población general. Esto podría significar que la respuesta inmune normal en general inhibe el crecimiento de estos tumores. Es más probable que esta incidencia tumoral aumentada en transplantados renales y cardíacos inmunosuprimidos se deba a una disminución de la respuesta inmune a un nivel que resulte estimulatorio. Otros tumores, como los de mama, tienen, en contraste con los de piel, una incidencia inferior a la esperada en pacientes inmunodeprimidos. Se postula que estos tumores tienen en promedio una baja inmunogenicidad que induce una respuesta inmune igual o inferior a la requerida para una proliferación tumoral óptima; reducir aun más el nivel de respuesta inmune, como ocurre en transplantados renales o cardíacos, lleva a una incidencia tumoral disminuida. Predecimos que en estas enfermas, un adyuvante o una vacuna podría a veces acelerar en vez de inhibir el crecimiento tumoral.

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In research the facts never *"speak for themselves". They must be selected, marshaled, linked together, and given a voice. Obviously research is not an end in itself. The day comes when the pleasures and the drudgery of the detective hunt are over and the report must be written. At that point, fit expression no longer appears as a mere frill added to one's accumulation of knowledge. The expression* is *the knowledge. What is not properly presented is simply* not present *- and its purely potential existence is useless.*

En investigación, los hechos *nunca* "hablan por sí mismos". Deben ser seleccionados, elaborados, interconectados, y valorados en su justa medida. Obviamente la investigación no es un fin en sí mismo. El día llega en que tanto el placer como la rutina del trabajo de "detective" llega a su fin y el manuscrito debe ser escrito. En ese momento, la expresión correcta no es un mero adorno de los resultados. La expresión *es* el nuevo conocimiento. Lo que no se expresa adecuadamente simplemente *no está presente* - y su existencia puramente potencial no sirve de nada.

Jacques Barzun & Henry F. Graff

The modern researcher. New York: Harcourt Brace Jovanovich, Inc., 1977, p X