genous sulfhydryl for mitochondrial protection.

O. danica resembles thyrotoxic animals in responding to fatty acids(15). Although linoleic acid was not active. Tween 85 permitted growth. The activity of Tween 85, contrasted with the inactivity of oleate and linoleate, raises the question of how much of the activity of Tween 85 is attributable to compounds other than the pure fatty acids used here. Since glutathione affects redox enzymes, the action of Tween 85 and glutathione may relate to their ability to maintain a redox potential favorable for mitochondrial function. Whether this involves the redox behavior of B_{12} itself remains to be seen. In Tetrahymena, a similar reversal of alkoxy-2,6-diaminopyrimidine inhibition has been shown for soy bean lecithin(17); the active components of the lecithin were not identified. The authors suggest that the lack of specificity of the lipid effect may mean that the inhibitors studies act as uncoupling agents. Such a possibility would apply with special force to thyroactive compounds(18).

Summary. The growth inhibition induced by thyroactive compounds on vit. B_{12} and non B_{12}-requiring microorganisms was studied. Thyroactive compounds were inactive for the B_{12}-requiring mutant *Escherichia coli* 113-3, *Lactobacillus leichmannii,* and *Euglena gracilis,* and for their non B_{12}-requiring counterparts. Only *Ochromonas malhamensis,* a B_{12}-requirer, and *Ochromonas danica,* a non B_{12}-requirer, were inhibited. Vit. B_{12} overcame the antagonistic action of the thyroid hormones for *O. malhamensis,* but not for *O. danica* where reduced glutathione and Tween 85 overcame the growth inhibition.

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Evidence for Immunization of F_1 Hybrid Mice Against Parental Transplantation Antigens. (26812)

GUSTAVO CUDKOWICZ (Introduced by A. C. Upton)
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*

Generally implicit in transplantation experiments with mouse tissues has been the assumption that grafts of parental origin are nonantigenic to F_1 hybrid recipients(1-3).

* Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.

This assumption, however, has been criticized on theoretical grounds(4-7) and appears to be at variance with certain experimental findings(8-12). This report presents preliminary results which are considered highly significant in that they support the belief that F_1 hybrid mice may be actively immunized against parental transplantation antigens.

Methods. Young adult (3- to 5-months old) hybrid recipients and parental donors of the following strains were used: (C57BL/Cum × 101/Cum)F_1, (C3H/Anf Cum × 101/Cum)F_1, (BALB/c Cum × A/He Cum)F_1, C57BL/Cum, C3H/Anf Cum, BALB/c Cum, A/He Cum, and 101/Cum; these are hereafter designated as $B1F_1$, $C31F_1$, CAF_1, C57, C3H, BALB/c, A, and 101, respectively. In every case, donor and recipient were of the same sex so that sex-determined histoincompatibility was avoided. Recipients were exposed to lethal whole-body irradiation as follows: strains $B1F_1$ and $C31F_1$ were given 900 r of X rays, and strain CAF_1, 800 r. The dose rate was approximately 85 r/minute; TSD, 93.5 cm; 300 kvp; 20 mA; 4.78 mm of Be inherent filtration and 3 mm of Al added filtration; hvl, 0.55 mm of Cu.

Hybrid mice were "immunized" against parental transplantation antigens by intraperitoneal inoculation of 20×10^6 nucleated viable parental spleen cells 30 and 15 days before irradiation. The parental spleen cells were obtained by teasing the spleen in Tyrode's solution with forceps and needles. The cell suspension was then passed through a stainless steel screen (200 mesh/inch) and the nucleated cells were counted in a hemocytometer.

Hybrid mice were also "immunized" by grafting of parental skin, the irradiation being carried out 100 days later without removal of the skin graft. Donor skin was taken from the ears and grafted onto the dorsal area of F_1 hybrid recipients by the method of Billingham and Medawar(13). The skin of the ear was used to avoid, insofar as possible, transplantation of dermal elements and included lymphoid cells. In every experiment, aliquots of a given parental marrow preparation were injected into each of the following groups of 10 recipients: (a) "nonimmunized" F_1 hybrids, (b) F_1 hybrids "immunized" against the parental donor strain, (c) F_1 hybrids "immunized" against the reciprocal parental strain. In addition, isologous marrow was given to F_1 hybrids "immunized" against one of the parental strains. The experiments were repeated to build up groups of 40 mice each, unless otherwise stated. Marrow cells were extruded from the femoral marrow cavity in Tyrode's solution by means of a syringe fitted with a 24-gauge needle. Recipients were inoculated with 10^7 nucleated cells by tail vein within 3 hours after irradiation. Characterization of hemoglobin type(14) in the $B1F_1$ recipients was carried out to ascertain whether donor type C57 red blood cells were present. Although irradiated marrow recipients were not serially sacrificed to follow pathological changes, dying mice in some of the experimental groups were sacrificed *in extremis* 20-90 days after treatment, and the following tissues were removed for histological examination: bone marrow from a long bone and the sternum, spleen, lymph nodes, thymus, Peyer's patches, intestine, liver, and kidney. Tissues of 16 B1F, 10 $C31F_1$, and 10 CAF_1 "immunized" mice were studied.

Results. In all donor-host combinations, the marrow protected against early lethality. Secondary mortality, however, occurred during the second and following months, varying in relation to strain and pretreatment of recipients (Fig. 1-3). In nearly all instances, secondary morbidity and mortality in F_1 recipients of parental marrow was accentuated by "immunization" against the donor strain; *i.e.,* by the preirradiation inoculation of parental lymphoid cells or grafting of parental skin. This effect was most marked in those strain combinations having parental H-2 histocompatibility differences proved to be significant for marrow transplantation(15), *i.e.,* the $B1F_1$ and the $C31F_1$ (Fig. 1 and 2). In the CAF_1 hybrid, preinoculation of A strain spleen cells increased secondary mortality only slightly (Fig. 3) but greatly increased the severity and frequency of secondary morbidity, *i.e.,* all of such recipients suffered marked weight loss, ruffling of fur, and epilation, changes which persisted until the end

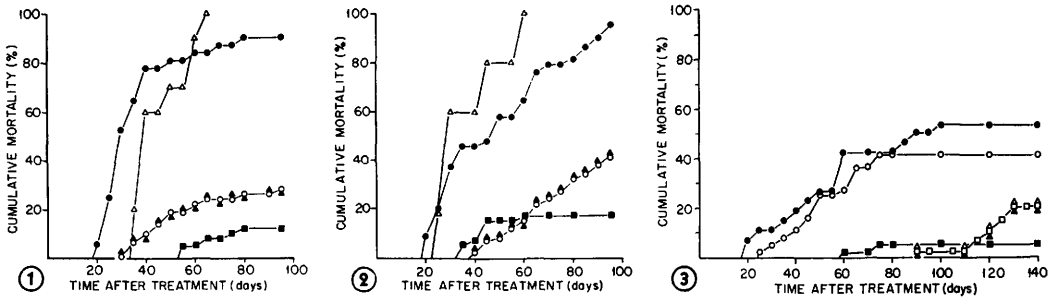

FIG. 1. Cumulative mortality of lethally irradiated $C31F_1$ mice after infusion of C3H or isologous marrow. Recipients were "immunized" against either parental spleen cells or skin. No. of animals in parentheses. ○ "Nonimmunized"; C3H marrow (40). ● C3H spleen; C3H marrow (40). △ C3H skin; C3H marrow (10). ▲ 101 spleen; C3H marrow (40). ■ C3H spleen; $C31F_1$ marrow (40).

FIG. 2. Cumulative mortality of lethally irradiated $B1F_1$ mice after infusion of C57 or isologous marrow. Recipients were "immunized" against either parental spleen cells or skin. No. of animals in parentheses. ○ "Nonimmunized"; C57 marrow (40). ● C57 spleen; C57 marrow (40). △ C57 skin; C57 marrow (10). ▲ 101 spleen; C57 marrow (40). ■ C57 spleen; $B1F_1$ marrow (40).

FIG. 3. Cumulative mortality of lethally irradiated CAF_1 mice after infusion of parental or isologous marrow. Recipients were "immunized" against either parental spleen cells or skin. No. of animals in parentheses. ○ "Nonimmunized"; A marrow (40). ● A spleen; A marrow (40). ■ A spleen; CAF_1 marrow (40). □ "Nonimmunized"; BALB/c marrow (40). ▲ BALB/c spleen; BALB/c marrow (40). △ BALB/c skin; BALB/c marrow (10).

of the observation period; in contrast, these symptoms were transient and mild in corresponding "nonimmunized" recipients of the same strain. In CAF_1 recipients of BALB/c marrow, secondary morbidity and mortality were minimal and unaffected by "immunization" against BALB/c cells (Fig. 3). In no combination did preirradiation inoculation of parental spleen cells homologous to the marrow donor strain affect the response of the recipients (Fig. 1-3). Hemoglobin in the peripheral blood of all 60-day and 80-day surviving $B1F_1$ recipients, whether "immunized" or not, was of donor type (70-100% of total Hb), indicating that the grafted erythropoietic cells were functional during the 80 days following parental marrow transplantation.

Histological preparations showed that the femoral and sternal marrow cavities were filled with nucleated cells, the marrow being at various stages of regeneration depending on the length of time elapsed from treatment. In no graft-host combination, even in "immunized" groups, was marrow necrosis noted. Lymph nodes and Peyer's patches of $B1F_1$ chimeras showed severe pathological changes similar to those described in the foreign bone marrow reaction(16), with atrophy and destruction of germinal centers, granulomatous proliferation of reticuloendothelial cells, and fibrosis; no difference was noted between "immunized" and "nonimmunized" recipients. The lymphoid tissues of the $C31F_1$ and CAF_1 chimeras showed mild changes, mainly atrophic in nature. They eventually recovered. Similarly, in these lymphoid tissues no differences were noted between "immunized" and "nonimmunized" recipients.

Discussion. The accentuation of secondary morbidity and mortality in irradiated F_1 hybrid recipients of parental marrow by pregrafting of donor-strain spleen cells or skin may be interpreted to mean that the grafting immunized against the donor marrow antigens. That this effect was immunological in nature is suggested by the correlation between its severity and the degree of histoincompatibility between the parental strains in question and by the lack of effect when nonspecific spleen cells (*i.e.*, cells other than those of the marrow donor strain) were preinoculated. Supporting data have been obtained in other experiments in which injection of isologous F_1 immunologically competent cells along with parental marrow into lethally-irradiated F_1 recipients leads to enhanced mortality, results suggesting that the F_1 hybrid cells reacted against parental mar-

row cell antigens(10,12). Also compatible with such an interpretation is the observation that isologous F_1 spleen (Cudkowicz, unpublished data) or marrow(17) cells inhibited the graft-versus-recipient reaction resulting from inoculation of parental liver cells into sublethally irradiated F_1 recipients (17).

The absence of enhanced morbidity and mortality in CAF_1 mice inoculated with BALB/c spleen cells and then given BALB/c marrow is tentatively ascribed to the lack of strong BALB/c histocompatibility antigens against which the CAF_1 hybrid can react; *i.e.*, the BALB/c is H-2^d in histocompatibility phenotype and possesses no major transplantation antigens foreign to the A strain (18,19).

Since the marrow was cellular and the circulating hemoglobin of donor type in moribund chimeras, death could not have resulted from rejection of the grafted parental marrow cells. The mechanism by which pregrafting accentuated secondary disease remains, therefore, to be elucidated. Death has been repeatedly observed, however, without necrosis of the marrow and spleen red pulp in parent-to-hybrid chimeras(9,20-24). In most of these cases the cellular and viable-appearing marrow was of host type, yet death was nevertheless ascribed to graft-versus-host immunological reactions; hence, other lesions resulting from *in vivo* antigen-antibody reactions are implicated and remain to be defined.

Enhanced secondary morbidity and mortality in the "immunized" F_1 chimeras might conceivably be attributed to aggravation of the graft-versus-host reaction by the inoculated parental lymphoid cells. This interpretation fails, however, to account for the similar effect of simultaneous injections of F_1 cells along with the parental marrow, and the lack of the effect in CAF_1 mice inoculated with BALB/c lymphoid cells. Furthermore, "immunization" of $B1F_1$ and $C31F_1$ hybrids could be accomplished just as effectively by skin grafting 100 days before irradiation as by inoculation of spleen cells. It is, therefore, inferred that the "immunizing action" did not require injection of viable donor-type antibody-forming cells and that it was not tissue-specific. "Immunization" has thus far failed to cause rejection of parental skin grafts in the strain combinations mentioned in this paper (Cudkowicz, unpublished data), suggesting that hemopoietic or lymphoid cells grafted into irradiated recipients may constitute a more sensitive indicator of histocompatibility differences than skin grafted onto unirradiated hosts.

There is now no direct evidence in the literature for active immunization of F_1 hybrid mice against parental transplantation antigens(7), the observed facts fulfilling generally the "one-autosomal gene, one antigen" theory. The occurrence of hybrid-anti-parent reactions, such as reported here, implies that parental antigen(s) are lacking in the hybrids between 2 inbred lines. Nevertheless, genic or allelic interactions in the inheritance of mouse histocompatibility factors have been postulated by many investigators on theoretical grounds or to explain certain experimental results(5-7,11,25) and have been demonstrated in species other than the mouse(7, a review). Their discovery in the mouse is, therefore, not altogether revolutionary.

Summary. 1. Previous grafting of parental spleen cells or skin accentuated secondary disease in F_1 hybrid mice subsequently irradiated and inoculated with marrow from donors of the same parental strain. 2. The intensity of the effect varied in relation to the extent of H-2 histocompatibility differences among the 4 strain combinations tested. 3. The effect is ascribed to "immunization" of the F_1 hybrid against parental tissue antigens. It is, therefore, inferred that parental histocompatibility alleles may not necessarily be expressed as codominants in F_1 hybrid mice.

Addendum. After submission of the manuscript, additional experiments showed that when the CAF_1 hybrid was "immunized" against A spleen cells by *three,* instead of two, inoculations at 30, 20, and 10 days prior to marrow grafting, respectively, secondary mortality *i.e.,* between 20-90 days) was enhanced to an extent comparable with that noted in the other strain combinations tested. However, the same treatment with BALB/c cells was not followed

by increased secondary mortality. These results indicate, therefore, that A strain parental tissues are also capable of "immunizing" F_1 hybrid mice.

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Effect of Stress on Potassium Content of Rat Brain. (26813)

F. B. BENJAMIN, JOAN N. ANASTASI AND W. M. HELVEY
Life Sciences, Republic Aviation Corp., Farmingdale, N. Y.

Previously it was shown that various types of injurious stimuli, when applied to peripheral tissues, cause a release of intracellular potassium(1), and that this release is prevented by analgesic agents(2). Adriani(3) suggests that process of excitation and inhibition in the C.N.S. may parallel those of peripheral mechanisms. A specific relationship between C.N.S. excitability and potassium (K) is indicated by the work of Ghosh and Quastel(4), Himwich(5), and Tsukeda (6). A number of workers found a low serum K during anesthesia(7,8,9). The present investigation represents an attempt to determine whether release and uptake of K by brain cells may be one aspect of the C.N.S. phase of the general stress response.

Methods. Rats were exposed to 3 different types of stress conditions: 1. (a) Anoxia was produced by evacuating a bell jar to an air pressure of 225 mm Hg. This was maintained for 120 minutes. (b) After return to ambient pressure, the animal was immediately decapitated. The brain was peeled out and washed in K-free Ringer solution. The 2 hemisections were separately macerated with fine sand, pestle and mortar. Potassium (K) content was determined from flame-photometric analysis of the watery extract and expressed as microequivalents per gram of dry tissue weight.

2. Heat stress was produced by putting a rat in a hot chamber at 42°C. The rat was maintained in the chamber until there were signs of impending collapse (air hunger). This took between 17 and 32 minutes with a