mune macrophages on target cells was further demonstrated by their failure to form plaques on monolayers of HeLa cells and their capacity to form plaques on monolayers of L cells from C3H mice. Both C3H mice and A/Jax mice possess antigen or antigens determined by the *K* allele of the $H_2$ locus, an allele which is lacking in C57B1/6K mice.

The possibility that control "nonspecific target cells" might be injured if placed in close association with interacting immune cells and specific target cells was explored by depositing immune cells at foci on a mixed monolayer consisting of HeLa and L cells in ratios of 5 : 1, 4 : 1, 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5. The plaque of partial clearing shown in Fig. 1*B* was produced on a monolayer comprised of equal numbers of L cells and HeLa cells. The plaque which formed in 24 to 48 hours appeared to have resulted from the complete destruction of the L cells without injury to the HeLa cells. Whereas essentially all of the L cells in the foci were killed regardless of the ratio of HeLa cells to L cells, the HeLa cells did not show evidence of injury. The transparency of the plaques correlated directly with the proportion of L cells present in the monolayer. Grossly visible plaques were evident at ratios of HeLa cells to L cells between 2 : 1 to 1 : 5.

The influence of increasing the ratio of immune cells to target cells by holding the immune cells constant and varying the density of the monolayers of A/Jax fibroblasts and monolayers of L cells was investigated. The results showed that the target cells in monolayers of low density were rapidly destroyed during the period extending from 1 to 6 hours. This rapid destruction of target cells in monolayers of low density was presumed to take place because of the large numbers of immune cells which made contact with the target cells in such preparations. More than 95 percent of the adhering cells were macrophages. Whereas only an occasional lymphocyte was adherent to the target L cells, most lymphocytes were free. Contact between macrophages and target cells is shown in Fig. 2.

The following additional observations have been made. Plaques are formed on monolayers of A/Jax fibroblasts by: (i) peritoneal cells derived from C57B1/6K animals immunized by the intraperitoneal injection of A/Jax spleen cells, (ii) peritoneal cells derived from C57B1/6K animals immunized subcutaneously with SaI, (iii) immune macrophages purified by the iron magnet method of Rous and Beard containing 96 percent macrophages and 2 percent lymphocytes (*9*). Lymphocyte-rich preparations obtained from ascitic exudates of SaI-immunized animals by repeated slow-speed centrifugation in the standard tissue culture medium destroyed a limited number of target cells in monolayers of A/Jax fibroblasts but did not produce visible plaques. The final lymphocyte-rich supernatant fluid contained 73 percent lymphocytes and 25 percent small macrophages.

In none of the cytologic studies was there any evidence that target cell injury resulted from phagocytosis by immune cells.

The results establish that peritoneal macrophages from C57B1/6K mice immunized with Sarcoma I ascites tumor produce specific immunologic injury of the target cells, macrophages and fibroblasts, by a nonphagocytic mechanism which apparently does not depend on humoral antibody, but instead demands contact between immune cell and target cell. The phenomenon probably involves antigen(s) determined by the K allele of the $H_2$ locus and is distinct from the opsonin-induced phagocytic destruction of target cells by macrophages studied by Old, Boyse, Bennett, and Lilly (*10*).

That adherence, the first step in the interaction of the immune cell and the target cell, is specific leads us to favor the concept that this may be the only specific component in the interaction and that by inducing intimate contact of cell membranes it may promote exchange of cytoplasmic materials which secondarily leads to cell injury.

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## Fetal Death from Nicotinamide-Deficient Diet and Its Prevention by Chlorpromazine and Imipramine

Abstract. *Feeding a diet deficient in nicotinamide, nicotinic acid, and tryptophan to pregnant rats causes death and resorption of all fetuses. This effect can be prevented by administration of either chlorpromazine or imipramine. Analysis of maternal liver at the time of fetal resorption indicates that the observed effects may be mediated through modification of the concentration of the pyridine nucleotide coenzymes.*

Fetal abnormalities, fetal death, and fetal resorption have been produced by diets deficient in any one of several different vitamins (*1*). However, the effect on fetal development of feeding the mothers diets deficient in nicotinamide does not appear to have been reported, although 6-aminonicotinamide, a nicotinamide antagonist, has been shown to be teratogenic (*2*).

We have now found that feeding pregnant rats a diet deficient in all three known sources of nicotinamide—nicotinamide, nicotinic acid, and tryptophan—from the first day after conception, resulted in the loss of fetal viability (Table 1).

The increase in the concentration of nicotinamide adenine dinucleotide (NAD) which occurs in response to administration of nicotinamide, is maintained for a longer period of time if animals are treated first with either of the tranquilizers chlorpromazine or reserpine (*3*). Since the mechanism by which the tranquilizers cause the increase of NAD to be maintained is being investigated in this laboratory (*4, 5*), it was of interest to determine whether chlorpromazine would manifest a "nico-

tinamide-sparing" effect in animals on a nicotinamide-deficient diet.

Administration of chlorpromazine did, in fact, permit the survival of most fetuses in pregnant animals fed the nicotinamide-deficient diet (Table 1). The antidepressant drug, imipramine, a close structural analog of chlorpromazine, also enabled most of the fetuses to survive (Table 1). Iproniazid, another antidepressant, was somewhat less effective, doses of up to 80 mg per kilogram of body weight enabling only about 25 percent of the fetuses to survive. Administration of nicotinamide to pregnant animals fed the deficient synthetic diet also maintained fetal viability, an indication that the effect of the synthetic diet was due to a lack of nicotinamide and the other precursors necessary for pyridine nucleotide coenzyme synthesis. Pregnant rats fed the deficient diet from day 2 through day 13 of pregnancy delivered viable offspring spontaneously at term if treated with chlorpromazine or imipramine from day 4 through day 12 of pregnancy (Table 2).

The concentration of NAD in maternal liver was examined at the time of fetal resorption 12 days after conception. The results show that the nicotinamide-deficient diet resulted in a decrease in the concentration of NAD (Table 1). Moreover, the concentration of NAD in the livers of pregnant rats fed the deficient diet was significantly higher in those animals treated with chlorpromazine or imipramine, in doses permitting fetal survival, than in the nontreated animals. Thus, in animals fed the unsupplemented deficient diet, the fetuses failed to survive, and the NAD concentration was low; on the other hand, treatment with chlorpromazine or imipramine prevented both the fetal morbidity and the decrease in NAD. The possibility clearly exists that the higher concentrations of NAD in the drug-treated animals are responsible for the survival of the fetuses.

An interesting contrast to our results is provided by the observation that chlorpromazine and pentobarbital do not prevent the fetal morbidity and teratogenicity induced by injection of salicylate (*6*). As might be expected, teratogenicity brought about by environmental stress could be reduced by the tranquilizer and barbiturate.

It has been suggested that the effect of chlorpromazine on the amount of the pyridine nucleotides in liver is mediated through a modified secretion of hormones of the anterior pituitary (*5*), since hypophysectomy caused an increase in liver NAD under the same conditions as the administration of chlorpromazine. As a working hypothesis, it is proposed that the capacity of chlorpromazine and imipramine to permit fetal survival in nicotinamide-deficient rats is also mediated through the endocrine system, possibly through hormonal control of pyridine nucleotide concentration.

Table 1. Fetal viability and NAD levels in pregnant rats. The *P* values compare the mean NAD value of the indicated group with that of the deficient group given saline. Virgin female rats (190 to 210 g) were placed in cages with males until conception occurred. Conception was assumed to have occurred when cornified cells and spermatozoa appeared in the vaginal smear, and this was termed day 1 of pregnancy. Animals were fed a diet deficient in nicotinamide, nicotinic acid, and tryptophan from day 2 through day 13 of pregnancy (*7*). Test agents, given from day 4 through day 12 of pregnancy, were administered by stomach cannula. Animals were killed on day 13 of pregnancy. The uteri were examined for the number of implantation sites and for fetal viability under an Edualite macroscope (magnification ×5). The concentration of liver NAD was determined by minor modification (*5*) of the procedure described by Ciotti and Kaplan (*8*) for the Racker alcohol dehydrogenase assay (*9*).

| Diet* | Test agent | No. of pregnant animals | No. of implantations | Percentage of viable fetuses | Liver NAD ($\mu$g/g ± SE) | *P* |
|---|---|---|---|---|---|---|
| | | | *Experiment 1* | | | |
| N | Saline | 25 | 235 | 94.1 | 448.9 ± 11.52 | <0.001 |
| D | Saline | 24 | 229 | 3.9 | 301.4 ± 8.23 | |
| D | Chlorpromazine (20 mg/kg) | 23 | 214 | 81.4 | 372.5 ± 12.20 | <0.001 |
| D | Imipramine (50 mg/kg) | 22 | 200 | 72.5 | 377.0 ± 13.01 | <0.001 |
| | | | *Experiment 2* | | | |
| N | Saline | 11 | 99 | 98 | 420.2 ± 13.1 | <0.001 |
| D | Saline | 12 | 127 | 0 | 326.4 ± 11.8 | |
| D | Imipramine (50 mg/kg) | 10 | 84 | 95 | 392.2 ± 22.3 | <0.01 |

* N, normal diet; D, deficient diet.

Table 2. Birth of living offspring in nicotinamide-deficient rats.

| Test agent | Rats with viable fetuses* | Total fetuses born (No.) | Fetuses born alive | |
|---|---|---|---|---|
| | | | No. | Av. body wt. (g) |
| | *Normal diet* | | | |
| Saline | 10/13 | 89 | 89 | 5.65 |
| | *Deficient diet* | | | |
| Saline | 0/12 | 0 | 0 | |
| Chlorpromazine (20 mg/kg) | 9/12 | 85 | 84 | 5.53 |
| Imipramine (40 mg/kg) | 9/11 | 73 | 64 | 5.84 |

* Ratio of the number of rats with viable fetuses to the total number of mated rats.

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