Influence of Host Protein Nutrition on the Response of Various Tumors to Ionizing Radiation* (32978)

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Investigations by Elson and Lamberton (1) and Devik *et al.* (2) have indicated that the response of a transplantable Walker rat carcinoma 256 to X-radiation is influenced by the level of dietary protein used to maintain the host animal. Because of the disparity in the genetic constitution of the tumor and the random-bred hosts used in the above studies, they do not provide an adequate model for evaluating the influence protein has on the response of spontaneous tumors to radiation (3,4). However, the inherent technical difficulties in performing analogous experiments in animals with spontaneous tumors are obvious. Therefore, to avoid some of these difficulties, the present studies were done to ascertain to what extent the level of dietary protein influences the response to ionizing radiation of transplantable syngeneic tumor in rats, a C3H tumor in CBF_1 hybrid mice, and an allogeneic tumor in random-bred mice. The results obtained indicate that the level of dietary protein is an important factor in the elimination of the tumors in random-bred mice through radiation therapy, but in contrast, the level of host protein nutrition in either the syngeneic tumor in rats or the C3H tumor in F_1 hybrid mice was ineffective in assisting radiation to cure any of the tumors. Nevertheless, the level of protein fed influenced the host survival time following radiotherapy.

Materials and Methods. Animals. The mice used in these studies were F_1 hybrids of C3H/Wr and C57BL6/Wr strains obtained from our breeding colony. These animals were not more than five generations removed from a single mating pair in the nucleus stock of their respective strains. Random-bred ICR/Ha mice and inbred male rats of the W/Fu strain were purchased from the A. R. Schmidt Company, Madison, Wisconsin.

Tumors. The C3H mouse tumor used in the F_1 hybrid host studies was a mammary adenocarcinoma MTG-B (5). Sarcoma 180 was the allogeneic mouse tumor employed in the random-bred ICR/Ha mice. The syngeneic rat tumor employed, MT/W9D, is a mammary adenocarcinoma (hormone insensitive) that was kindly supplied by Dr. U. Kim (Roswell Park Research Institute, Buffalo, New York). Transplant techniques used were similar to those described by Clifton and Draper (6). Each host animal received an injection of a suspension of fresh tumor cells in the lateral aspect of the right hind leg.

Diet. Diets containing different levels of protein (0, 8, 26, and 62% casein) were obtained from the GBI Corporation, Chagrin Falls, Ohio. The diets were made isocaloric by replacing the casein with suitable amounts of sucrose and dextrose. The 0% protein diet was supplemented by allowing the animals *ad libitum* access to a B vitamin mixture. The feeding regimes employed are described below in "*Results*".

Radiation. All mice were irradiated with a superficial X-ray machine operated at 140 kvp, 5 ma, with a half-value-layer of 3 mm of Al. A lead shield was used which permitted immobilization of the tumor-bearing leg and allowed a maximum dose of 0.05% to the shielded pelvic area. The following fractionated radiation schedule was utilized: 1000 R on the initial day of treatment, and 500 R on subsequent alternate days until a total of 4000 R was reached. In addition, for those animals receiving a total of either 4500 or 5000 R, 250 R/day was given on alternate days. The dose rate was 185 R/min.

All rats were irradiated with a 2000 Ci^{137} Ce source emitting 662-keV gamma

* This investigation was supported by Public Health Service Research Grant CA 08318, Center Grant CA 06295 from the National Cancer Institute and Grant T66I from the American Cancer Society.

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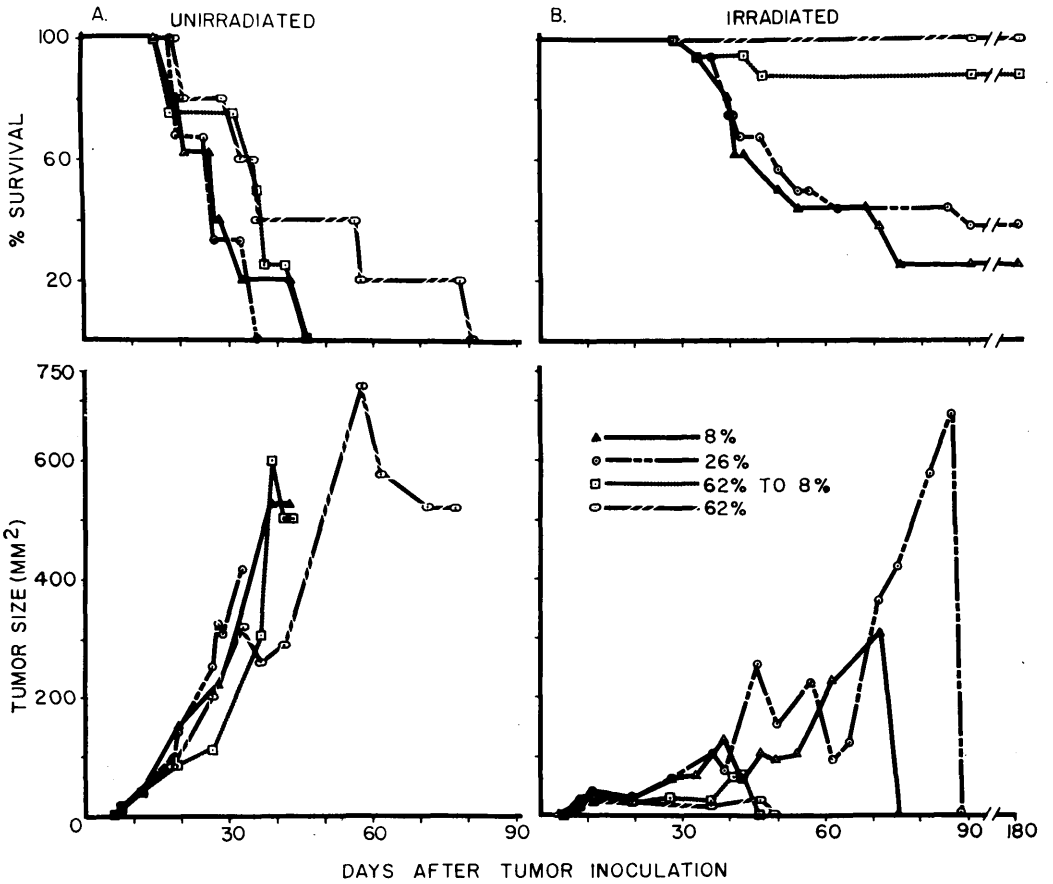

FIG. 1A. Growth of Sarcoma 180 tumor in ICR/Ha mice and the survival time of the hosts maintained on various levels of dietary protein. B. Response of tumor growth rate and survival of hosts maintained on the same dietary regime and treated with a fractionated course of X-radiation (total dose to tumor, 4500 R). Irradiation therapy begun on day 8 after tumor implantation and ended on day 24.

rays. A beam localizer head was used to limit the radiation to the tumor-bearing leg, and appropriate lead shielding protected the animal from radiation scatter. During irradiation the animals were restrained in lucite plastic tubes with the tumor-bearing leg extended through a hole in the tube and fixed in the radiation field. The distance from the radiation source to the medial aspect of the tumor-bearing leg was 35.9 cm and the dose rate was 60 R/min. The fractionated radiation schedule for the rats was as follows: 1000 R on the initial day of treatment followed by a 2-day treatment break, after which a second dose of 1000 R was administered. This was again followed by a 2-day treatment break after which five consecutive daily doses of 500 R were administered making a total dose of 4500 R delivered to the tumor at culmination of the radiation treatment.

Results. Sarcoma 180. The effect of different levels of host protein nutrition on growth of the Sarcoma 180 tumor and the subsequent influence of the dietary protein on the response of this tumor to a fractionated course of radiation are shown in Fig. 1A and B. In these experiments mice were pre-fed diets containing either 8, 26, or 62% casein for 2 weeks, at which time tumor cells were implanted. The tumors were allowed to grow untreated for an additional 8 days after implantation; then radiation was commenced

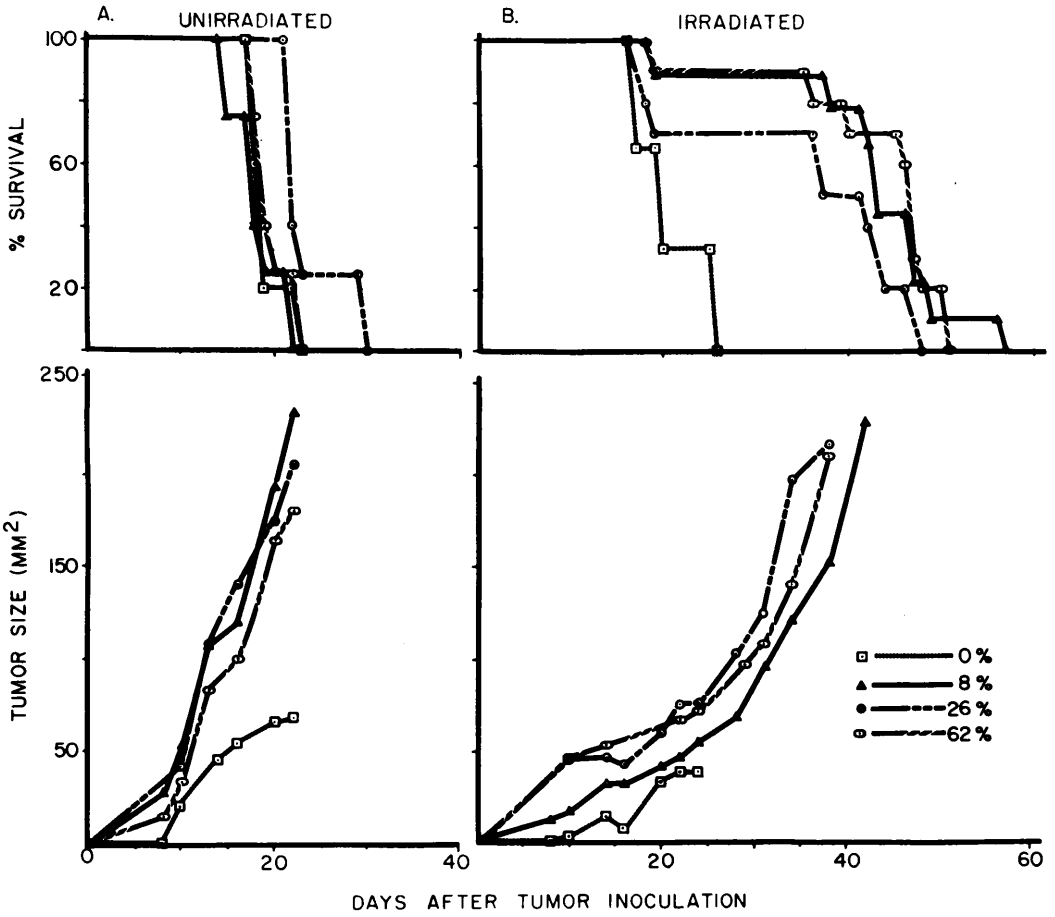

FIG. 2A. Comparison of the growth rate of MTG-B tumors and host survival time in mice maintained on different levels of protein nutrition. B. Response of tumor growth rate and host survival of CBF_1 hybrid mice fed different levels of dietary protein and given a fractionated course of X-radiation to the tumor of 5000 R. Irradiation started on day 8 after tumor implantation and stopped on day 28.

and continued until a total of 4500 R was delivered locally to the tumor. Concomitantly, half of the animals receiving the 62% casein diet were placed on the 8% protein diet, while the other groups of mice continued to receive their respective diets. Prior to irradiation the rate of tumor growth was essentially the same for all diet groups.

Since the tumors were quickly brought under control and their size remained essentially stationary during the course of irradiation, the different diets did not appear to modify early radiation response (Fig. 1B). However, at the termination of radiation all of the tumors in animals maintained on the high protein diet (62%) began to regress and were completely eliminated within 24 days after the cessation of irradiation. Similarly, 87% of the tumors in animals maintained on the high to low protein (62–8%) regime began to regress and were also completely eliminated within 24 days after termination of radiation therapy. In contrast, only 25% and 38% of the animals on the 8 and 26% protein diets, respectively, were able to completely eliminate their tumors following irradiation. All animals that eliminated their tumors survived and were free of tumor 6 months after implantation (Fig. 1B). Whereas all animals bearing unirradiated tumors

TABLE I. Mean Survival Time of Mice Bearing MTG-B Tumors.

	Protein fed (%)			
	0	8	26	62
Unirradiated	19.6 ± 0.9[a] (5)[b]	18.5 ± 1.4 (4)	24.3 ± 1.9 (4)	20.0 ± 1.1 (6)
Irradiated (4000 R)	27.2 ± 1.5 (18)	36.2 ± 3.5 (12)	42.3 ± 1.0 (14)	40.0 ± 0.9 (14)
Irradiated (5000 R)	21.0 ± 1.6 (6)	42.8 ± 3.5 (9)	35.1 ± 3.9 (10)	43.2 ± 3.1 (10)

[a] Days survival after tumor implantation ± SEM.
[b] No. of animals/group.

ultimately succumbed, only mice fed the high protein diet had an extended survival time when compared to the other diet groups (Fig. 1A).

Adenocarcinoma MTG-B. The effect of different levels of host protein nutrition on the initial growth rate of the MTG-B tumor and survival of its hosts are shown in Fig. 2A and the influence of dietary protein on tumor growth and host survival during the course of radiotherapy in Fig. 2B. In these studies mice were pre-fed diets containing 0, 8, 26, or 62% casein for 2 weeks prior to tumor inoculation. On the eighth day after transplantation, mice in each of the diet groups were divided into three subgroups. Tumors in each subgroup were treated with respect to irradiation as follows: (a) no irradiation, a fractionated course of radiation to a total dose of (b) 4000 R, or (c) 5000 R.

There was no effect of diets containing from 8 to 62% protein on tumor growth rate within the irradiated and unirradiated groups. However, tumors in animals fed the 0% protein diet grew more slowly, whether irradiated or not (Fig. 2A and B).

Unlike the Sarcoma 180 tumor-host system, the mean survival time of mice bearing unirradiated MTG-B tumors did not differ significantly as a result of the diet fed. Host survival was not affected by 5000 R irradiation to tumors in animals fed 0% protein; however, mice on the 0% protein diet treated with 4000 R survived longer (95% confidence limits) than similarly fed unirradiated animals (Table I). In addition, survival time of animals on 8 to 62% protein was extended by a course of either 4000 or 5000 R of fractionated radiation (Table I).

Adenocarcinoma MT/W9D. The rats were divided into three groups and pre-fed diets containing either 8, 26, or 62% of casein protein for 2 weeks prior to tumor inoculation. After implantation, the animals remained on their respective diets and the tumors were allowed to grow without treatment for either 10 or 11 days. After that time, the tumors, which ranged in size from 3 × 3 to 8 × 8 mm, were given 4500 R in a fractionated schedule. Initially both the 8 and 62% casein diets appeared to cause a slower tumor growth rate which was maintained until about day 15 after tumor implantation in the nonirradiated groups (Fig. 3A). In the irradiated groups the tumor growth curves in hosts fed either 8 or 62% protein diets first plateaued after radiation treatment began, then for a period of approximately 1 week the tumors regressed, finally reaching a point where they were no longer palpable. This condition also lasted for approximately 1 week after which they began to reappear (Fig. 3B). The tumors in the animals fed the 26% casein diets also regressed as a result of the radiation; however, they were palpable at all times during and after the course of radiation therapy. Furthermore, tumors in rats fed diets containing 26% protein reestablished their preirradiation growth patterns more rapidly than those growing in hosts fed the diets composed of either 8 or 62% casein (Fig. 3B). Tumor measurements were suspended 70 days after implantation because of tumor necrosis and the propensity of rats to eat the tumors of their cage mates. The mean survival time of host rats fed the three different protein diets is shown in Table II. On a 26% casein diet rats lived longer, with radiation, than rats in the other two diet groups (95% confidence limits). The mean survival

times of rats bearing unirradiated tumors did not differ significantly as a result of the diet fed. In addition, their tumors were larger at the time of death. This situation is in agreement with the MTG-B mouse tumor study.

Discussion. The influence of host protein nutrition on the response of a tumor to a fractionated course of radiation has been evaluated on the basis of the genetic relationship of the tumor to its host in both mice and rats. The parameters studied in evaluating the effects of host protein diet have been: (a) tumor growth, (b) tumor cure, and (c) host survival time. In earlier studies using an allogeneic rat tumor-host system others (1,2) have shown that the level of protein in the

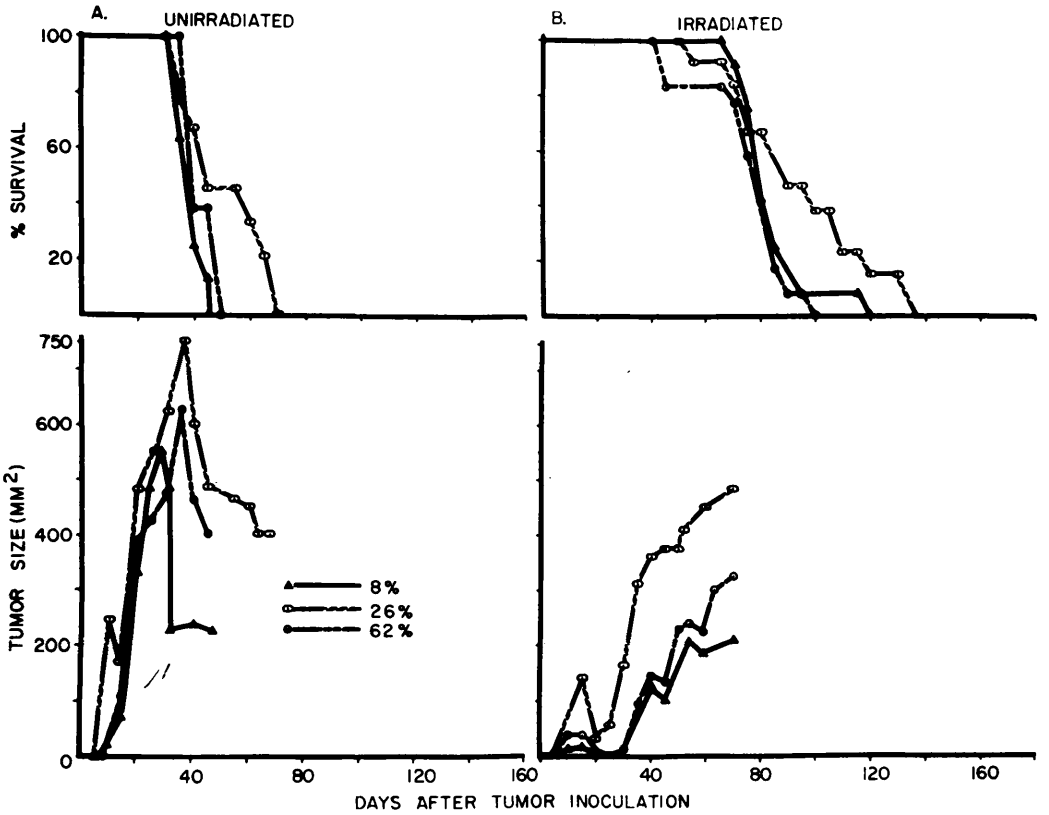

FIG. 3A. Comparison of the growth rate of MT/W9D tumors and host survival time in rats maintained on different dietary regimes. B. Tumor growth rate and host survival of W/Fu rats fed the same levels of protein in the diet with the tumor being subjected to a fractionated course of gamma-radiation to a total dose of 4500 R. Irradiation begun on day 10 after tumor implantation and ended on day 20.

TABLE II. Mean Survival Time of Rats Bearing MT/W9D Tumors.

	Protein fed (%)		
	8	26	62
Unirradiated	37.9 ± 1.9[a] (8)[b]	48.3 ± 4.8 (9)	40.9 ± 1.3 (8)
Irradiated (4500 R)	81.8 ± 3.4 (12)	94.4 ± 7.3 (13)	73.3 ± 4.7 (12)

[a] Days survival after tumor implantation ± SEM.
[b] No. of animals/group.

diet plays an important role in the initial tumor growth rate in enhancing host survival time and finally in the ability of X-radiation to produce tumor cures. We have performed similar studies using an allogeneic mouse tumor–host system, and our results agree except that initial rate of tumor growth was not affected by the lowest level of protein (8%) provided to the host mouse. This discrepancy may be rationalized either on the basis of a difference in the level of casein protein fed [8% in our study as compared to 5% for Elson and Lamberton (1)], or in inherent differences in the nutritional requirements of mice and rats for protein.

The experiments with the tumor in its F_1 hybrid host reveal that complete elimination of protein from the diet significantly retarded tumor growth. However, this seemingly beneficial effect is negated by the fact that the tumors in mice on the 0% protein diets did not respond to a fractionated course of radiotherapy. Therefore, though the tumors grew more slowly, probably because of insufficient protein reserves (7–10), host survival time was not increased by radiation as it was in similarly treated animals fed some protein. It should also be noted that contrary to the situation obtaining in the allogeneic Sarcoma 180–ICR/Ha system, there were no tumor cures at any level of protein nutrition alone or in combination with X-radiation in the MTG-B tumor–host system. Similarly, there were no tumor cures in the syngeneic rat system.

Host survival time was greater after irradiation in the 26% casein diet group than in the other diet groups. Furthermore, with irradiation all diet groups survived longer, indicating that radiation had a host-sparing effect when administered at that stage of tumor growth. Unpublished data (Yatvin), however, suggest that radiation given at a later stage does not have this salutary effect.

The fact that higher protein diets enhance survival of mice bearing Sarcoma 180 corresponds well with the findings of Allison and co-workers (7–10) using allogeneic tumor systems. These workers observed that feeding high casein diets or a methionine-supplemented casein diet favored the development of the carcass of the tumor-bearing rat, thereby reducing the depleting effect of the growing tumor, which resulted in a more favorable condition for host survival.

The varying responses to diet and radiation obtained in these different tumor–host systems can probably be explained on the basis of the genetic composition of the tumor with respect to its host, and the immunological consequences of these differences. The Sarcoma 180 is the most divergent genetically from its host, the MTG-B in its F_1 hybrid less so, and finally there is very little difference in the MT/W9D and its syngeneic host. Possible explanations for the different responses of these tumors to diet and radiation may be found in the studies of Graham and co-workers (11,12). These workers have noted that focal irradiation of a site previously injected with antigen enhances both local and circulating antibody response. Thus, one effect of high protein diet may be simply that it serves as a source of precursors for enhanced antibody production. Since greater tumor antigenicity would be expected where the greatest genetic differences obtain between the host and its tumor, it is therefore not unexpected that the Sarcoma 180 ICR/Ha system showed the most striking results. No cures were evident in genetically less divergent systems. Thus the increased survival time of both the irradiated F_1 hybrid hosts carrying the MTG-B tumor and the irradiated syngeneic rat hosts is due to direct radiation killing of tumor cells.

These experiments are preliminary; however, future experiments designed to clarify the effect of the host's nutritive state on the response of a transplantable tumor to radiation will consider the following: (i) protein reserve, (ii) direct radiation cell killing, and (iii) enhanced immune response following local irradiation.

Summary. The response of various transplantable tumors to a fractionated course of radiation therapy has been studied in host mice and rats fed different levels of dietary protein. The parameters employed in evaluating the effect of diet have been: (a) tumor growth, (b) tumor cure, and (c) host survival time. The tumor used in these studies most

genetically divergent from its host (Sarcoma 180) showed the most striking response to diet and radiation. Tumors less divergent from their hosts were markedly less responsive. It is proposed that the varying responses obtained in these studies can be explained on the basis of the protein reserves of the host, and the genetic composition of the tumor with respect to its host and the resulting immunological consequences.

We wish to thank Dr. Kelly H. Clifton for his interest and advice, and H. Maseehuddin for technical assistance.

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Received Jan. 16, 1968. P.S.E.B.M., 1968, Vol. 128.

Specific Response of the Immunoglobulins to Rubella Infection* (32979)

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The teratogenic consequences of rubella infection early in pregnancy are well known. However, experience with the rubella epidemic in 1964 revealed unexpected virologic and serologic consequences of maternal infection. Despite the fact that the rubella syndrome was caused by maternal infection prior to the immunologic maturity of the fetus, serologic immunity rather than tolerance was observed in older children with the syndrome (1,2). Furthermore, rubella virus was found to persist in the affected infants for many months despite the presence of serum antibodies (3–5). Attempts to equate the presence of rubella antibodies in these infants with an active immune response by means of conventional serology was complicated by the presence of passively acquired maternal antibodies of the IgG variety which persist for several months. Since IgA and IgM do not cross the placental barrier readily, the association of antibody activity within the IgM and /or IgA class of globulins of the infant's serum would indicate active immunity. The following studies were carried out in order to characterize the classes of immunoglobulin which responded specifically to rubella infection and to determine their chronology. Observations were also made regarding the classes of rubella antibodies in sera from "normal" newborns and infants with congenital rubella.

Materials and Methods. Rubella antibodies were demonstrated by the indirect fluorescent antibody method as described by Brown *et al.* (6). Whereas conventional methods of serum fractionation and antibody titration were cumbersome, this technique provided a rapid and reliable method for identification of the antigenic class of antibodies (IgG, IgA, or IgM) in whole serum which had combined

* Supported in part by PHS Grant AI-05876 from the National Institute of Allergy and Infectious Diseases, N.I.H., and in part by 5-TO-1 HD00057 from the National Institute of Child Health and Human Development, N.I.H.