Immunol. 90, 21 (1963).

4. Johnsson, T., Lavender, J. F., Hultin, E., and Rasmussen, A. F., Jr., J. Immunol. 91, 569 (1963).

5. Rasmussen, A. F., Jr., Hildemann, W. H., and Sellers, M., J. Natl. Cancer Inst. 30, 101 (1963).

6. Chang, S. S., Rasmussen, A. F., Jr., Bacteriol. Proc. 134 (1964).

7. Jensen, M. M. and Rasmussen, A. F., Jr., J. Immunol. 90, 17 (1963).

8. Pluznik, D. H. and Sachs, L., J. Natl. Cancer Inst. 33, 535 (1964).

9. Chirigos, M. A., Ann. N. Y. Acad. Sci. 130, 56 (1965).

10. Marsh, J. T. and Rasmussen, A. F., Jr., Proc. Soc. Exptl. Biol. Med. 104, 180 (1960).

11. Minster, J. J., Proc. Soc. Exptl. Biol. Med. 113, 377 (1963).

12. Ader, R. and Friedman, S. B., Psychosomat. Med. 27, 119 (1965).

13. Gottfried, B., Molomut, N., Proc. Intern. Congr. Cancer, 8th, Moscow, p. 220 (1962).

14. Mathtes, T., Proc. Intern. Congr. Cancer, 8th, Moscow, p. 218 (1962).

15. Marsh, J. T., Miller, B. E., and Lamson, B. G., J. Natl. Cancer Inst. 22, 961 (1959).

16. Smith, L. W., Molomut, N., and Gottfried, B., Proc. Soc. Exptl. Biol. Med. 103, 370 (1960).

17. Funk, G. A., Jensen, M. M., Proc. Soc. Exptl. Biol. Med. 124, 653 (1967).

Received Oct. 18, 1967. P.S.E.B.M., 1968, Vol. 127.

Friend Disease and a Tumor Variant in Hybrid (BDF_1) Mice* (32755)

A. HOWARD FIELDSTEEL, PETER J. DAWSON, AND JEAN SCHOLLER

Division of Life Sciences, Stanford Research Institute, Menlo Park, California, and Department of Pathology, University of Oregon Medical School, Portland, Oregon

Transplantable reticulum cell sarcoma variants of Friend virus leukemia reportedly have been induced only in inbred mice highly susceptible to Friend virus infection (1,2). However, only tissue from an occasional mouse with terminal Friend disease appeared capable of inducing these tumors. The tumors were readily transplantable in normal mice of the same inbred strain and invariably contained large quantities of virus. The latter has complicated *in vivo* antigenic studies because it rapidly induced fatal leukemia which is unrelated to the development of the tumor. One approach to the problem is to nullify the lethal activity of the virus. This could be accomplished if it were possible to induce a transplantable reticulum cell sarcoma in a host of low susceptibility to Friend disease. Adult BDF_1 mice, the offspring of Friend virus resistant C57BL/6 females and susceptible DBA/2 males, represent such a host because they are highly, but not totally, resistant to Friend disease (3). The unusual course of Friend disease, and the properties of an induced transplantable tumor in BDF_1 mice, are the subjects of this report.

Materials and Methods. The BDF_1 mice, obtained from Simonsen Laboratories, Gilroy, California, were young adult females. The BALB/c mice were 6- to 8-week-old animals of either sex from our own colony.

The strain of Friend virus, the method of preparing pools and the procedure for carrying out neutralization tests were the same as described previously (4). Virus was inoculated intraperitoneally (ip).

For the initial induction of tumors, spleen and liver from a Friend virus infected BDF_1 mouse were individually minced with iris scissors to a brei, suspended in Hanks' BSS and drawn into a 10-ml Luer-Lok syringe. The suspension was then expelled through an 18-gauge needle and the procedure repeated until a "heavy" homogeneous suspension was obtained. Initially BDF_1 mice were inoculated subcutaneously (sc) with 0.2 ml. When tumors appeared, they were removed aseptically and suspended in the same manner as the original tissue. Serial transplantation was carried out by either the sc or ip route.

For quantitative studies tumors were minced, washed 3 times with phosphate buf-

* Supported by USPHS Grant CA 07868 from the National Cancer Institute, and by Grant DR 6-928 from the Damon Runyon Memorial Fund.

TABLE I. Results of Inoculating BDF_1 Mice with Friend Virus.

Expt. no.	Passage history of virus[a]	Dose of virus ($-\log_{10} ID_{50}$)	Time to sacrifice (days)	Results (no. infected/total)	
				Spleen wt.[b]	Histologic[c]
1	SW11 BALB 4	3.6	21	9/56	54/56
2	SW11 BALB 7	3.7	33	0/10	9/10
3	SW11 BALB 7	3.7	498	1/10	8/10

[a] SW = no. of passages of virus in Swiss mice; BALB = no. of passages in BALB/c mice following Swiss mouse passage.

[b] Spleens weighing >0.5 gm were considered infected.

[c] Spleens examined histologically and those showing typical foci of proliferating reticulum cells were considered infected.

fered saline (PBS) to remove debris and red blood cells, and treated with 0.2% trypsin to produce a single cell suspension. This was diluted to desired concentrations of viable cells after enumeration in a hemocytometer in the presence of 0.05% trypan blue.

Results. Overt Friend disease was rarely observed in mature BDF_1 mice (Table I). Of 76 mice sacrificed from 21 to 498 days after inoculation only 10 (13%) had spleens weighing more than 0.5 gm. However, 71 (93%) of the mice had histologic evidence of disease. Of the group kept for 498 days, none of the spleens of the 9 survivors weighed more than 0.2 gm and there were no gross signs of leukemia. Microscopic examination of these spleens revealed foci of proliferating reticulum cells in 7, resembling in size and appearance those seen in mice killed 33 days postinoculation. The tenth mouse, obviously ill on the 300th day, was killed. It had massive hepatosplenomegaly, typical of Friend disease in highly susceptible mice. Histologically, there was subtotal replacement of spleen, liver, and bone marrow with actively dividing reticulum cells and scattered collections of erythroblasts.

Pieces of liver and spleen grafted sc into BDF_1 mice produced palpable tumors in 17 days. These were readily transplantable and were identified as reticulum cell sarcomas histologically identical to those previously established in BALB/c mice (2). Tumors derived from the original spleen and liver appeared to be similar in all respects and, therefore, only the one derived from liver was maintained. After 9 transplant generations sc in BDF_1 mice, the 50% tumor endpoint as determined by the Reed and Muench method was 6.8×10^2. However, the smallest number of cells that would regularly produce sc tumors in 100% of BDF_1 mice was 10^4 with an average time of onset of about 20 days. The tumors grew slowly, killing the mice 1–2 months after onset.

At the tenth transplant generation an experiment was carried out to determine whether the tumor was specific for BDF_1 mice and if it contained Friend virus. Two groups of BALB/c mice were inoculated ip with either 10^6 viable BDF_1 tumor cells washed 6 times in 50 ml of PBS, or a 20% cell-free extract of the same tumor. After 62 days none of the mice had developed tumors. However, 15 of 20 mice in the first group and 15 of 16 in the second had advanced Friend disease.

A series of transplantation experiments was undertaken to determine if the BDF_1 tumor was antigenic in isogeneic mice and, if so, whether the Friend virus associated with the tumor was responsible. Twenty BDF_1 mice were each inoculated 3 times ip with an average of 2.1×10^7 viable tumor cells treated with 10^{-3} *M* sodium iodoacetate according to the method of Apffel *et al.* (5). Inactivation apparently was not complete as 11 mice developed tumors and died between 20 and 74 days after the last inoculation. The 9 survivors and 20 previously uninoculated control mice of the same age were challenged sc with 1.6×10^4 viable tumor cells. All 20 controls developed tumors as compared with only 2 of the 9 vaccinated mice ($p < .001$).

Attempts were then made to protect BDF_1

TABLE II. Effect of Vaccination of BDF_1 Mice with Friend Virus on Subsequent Challenge with BDF_1 Tumor.

Treatment[a]	Number of cells used for challenge at day 50	Results (No. with tumors/ totals)	Totals
None	1.0×10^4	34/34	61/64
	4.8×10^3	27/30	
Normal BALB/c spleen extract	1.0×10^4	29/30	57/60
	4.8×10^3	28/30	
Friend virus	1.0×10^4	20/28	41/59
	4.8×10^3	21/31	

[a] Five doses of normal spleen extract or Friend virus over a 7-week period, the first and fourth doses given with Freund's adjuvant.

mice against the tumor by vaccination with live virus. No protection against sc challenge with 10^4 tumor cells could be demonstrated after vaccination ip with 5 weekly doses of $10^{3.9}$ ID_{50} of Friend virus.

In another experiment, groups of BDF_1 mice were vaccinated 5 times with either Friend virus or normal mouse spleen extract. However, the first and fourth inocula were suspended in an equal quantity of Freund's complete adjuvant. As a result of the inoculation of adjuvant, a small amount of ascitic fluid accumulated in the abdominal cavity of the majority of mice in each group. This was aspirated on day 49 after initial inoculation and the fluid from each group was pooled. After removal of cells and debris by centrifugation, it was inactivated at 56°C for 30 min. The following day mice from both groups plus a group of previously unvaccinated BDF_1 mice were challenged sc with either 4.8×10^3 or 1×10^4 viable BDF_1 tumor cells (Table II). Fifty-seven of 60 mice vaccinated with normal spleen plus adjuvant developed tumors compared with 41 of 59 vaccinated with the virus–adjuvant mixture. These results are highly significant ($p < .001$). The ascitic fluids from the vaccinated mice were tested for the presence of neutralizing antibody against Friend virus. Fluid derived from the group vaccinated with virus had a neutralization index of 570 while the fluid from the group vaccinated with normal spleen had no neutralizing antibody.

Discussion. While overt Friend disease was rarely induced in adult BDF_1 mice, 61 of 66 apparently healthy mice had microscopic evidence of disease. Although most of these mice were sacrificed 21–33 days after inoculation, at a time when 100% of BALB/c mice have fully developed disease (2), it is possible that some would have developed clinical disease. Of greater interest is the question of whether the resistance of BDF_1 mice was such that they would recover from the disease. This question was partially answered in the group held for 498 days after inoculation. The disease was nonprogressive and detectable only upon histological examination. Of the one mouse in this group which did develop the typical hepatosplenomegaly of Friend disease, transplantable tumors were induced from its organs. These were similar to those induced in susceptible mice in that they were host-specific, contained Friend virus, and were of the reticulum cell sarcoma type. The ease and rapidity with which the tumor was established was in striking contrast to our earlier experience with the highly susceptible BALB/c mice.

Although it was demonstrated that the tumor could induce transplantation resistance against subsequent challenge with it in isologous mice, it was not determined if the antigenicity of the tumor was due to new cellular antigen(s) or to Friend virus. The question remains why vaccination with virus in adjuvant conferred transplantation immunity while virus alone did not. Although the serum of mice vaccinated with virus alone was not tested, it is most unlikely that it did not contain neutralizing antibody against Friend virus. The fact that adjuvant had to be employed to protect mice against the tumor suggests that the preparations of Friend virus may have also contained, in addition to viral antigen, other weak antigen(s), and it was antibodies to the latter which conferred protection against the tumor. This possibility is supported by the finding that vaccination with similar virus preparations plus adjuvant will protect BALB/c mice against an iso-

geneic virus-free Friend virus-induced tumor.[1]

The nature of the antigen(s) involved has yet to be determined but these could include the soluble antigen reported by Stück *et al.* (6). For the present it does not seem unreasonable to suggest that malignant transformation of cells by Friend virus, a late phenomenon in Friend leukemia, is preceded by the formation of new cellular antigen(s). Extracts of infected spleens presumably would then contain this antigen(s), which, when administered with adjuvant, would invoke immunity against isogeneic tumor transplants.

Summary. Although only a small percentage of adult BDF_1 mice inoculated with Friend virus developed progressive Friend disease, a transplantable reticulum cell sarcoma was established from the one mouse with massive hepatosplenomegaly. Transplantation resistance to the tumor was induced by vaccination with either iodoacetate-treated tumor cells or Friend virus in adjuvant, but not with Friend virus alone.

[1] Fieldsteel, A. H., Dawson, P. J., and Scholler, J., submitted for publication.

1. Friend, C. and Haddad, J. R., J. Natl. Cancer Inst. 25, 1279 (1960).

2. Dawson, P. J., Fieldsteel, A. H., and Bostick, W. L., Cancer Res. 23, 349 (1963).

3. Wahren, B., J. Natl. Cancer Inst. 31, 411 (1963).

4. Fieldsteel, A. H., Dawson, P. J., and Bostick, W. L., Cancer Res. 23, 355 (1963).

5. Apffel, C. A., Arnason, B. G., and Peters, J. H., Nature 209, 694 (1966).

6. Stück, B., Old, L. J., and Boyse, E. A., Proc. Natl. Acad. Sci. U. S. 52, 950 (1960).

Received Oct. 18, 1967. P.S.E.B.M., 1968, Vol. 127.

Early and Late Effects of Growth Hormone on the Rat Diaphragm* (32756)

K. G. DAWSON AND J. C. BECK

McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

Investigations of the mode of action of growth hormone in the stimulation of protein synthesis have failed to completely elucidate this problem. In brief, studies utilizing the diaphragm of the hypophysectomized rat have shown that growth hormone stimulates the uptake of some but not all amino acids (1). When growth hormone is given *in vivo,* RNA polymerase is stimulated after 12 hours (2) and RNA synthesis is stimulated (3). However, no stimulation of RNA synthesis has been detected when the hormone is added *in vitro* (4,5), and inhibition of RNA synthesis with actinomycin does not prevent the *in vitro* stimulation of protein synthesis (4). It has therefore not been possible to identify the mechanisms involved in growth hormone stimulation of protein synthesis *in vitro.*

It is of significance that the reported studies have only assessed protein synthesis during incubations of two hours or less. Hjalmarson and Ahren (6) have shown that amino acid uptake is stimulated during the first two hours of exposure of the diaphragm to growth hormone, but after three hours, no stimulation is seen. Similar results have been independently noted in our laboratory (7), indicating that the short-term response of the diaphragm of the hypophysectomized rat differs significantly from the prolonged response. These findings, coupled with somewhat analogous findings by Goodman in studies on adipose tissue (8,9,10) prompted this investigation of the late effects of growth hormone on amino acid uptake and protein synthesis.

Materials and Methods. Fifty-gm rats obtained from the Charles River Breeding Laboratories and hypophysectomized at age 20 days, were killed by ether anesthesia and surgery, 2–3 weeks after operation. The intact hemidiaphragms were obtained with one of each pair serving as control, and incubation was carried out in the presence or absence of growth hormone (NIH-B7 and B12) in

* Supported by a Grant from the United States Public Health Service, No. HD-00511.