the tissues and lumen of irradiated intestine is considerably magnified in the presence of intestinal content. Consequently, lengthening of the isolated segment and shortening of the intestine in continuity should significantly improve post-irradiation survival. This is being investigated.

Summary. The abdomens of dogs with T-V fistulas of the jejunum were irradiated with 1,000 and 1,500r. The post-irradiation lesions in the small intestine in continuity were typical. Those in the fistulas, where intestinal content was absent, were considerably less intense.

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Antitumor Effects of Kethoxal-Bis(Thiosemicarbazone) and 6-Mercaptopurine in Neonatally Thymectomized Mice.* (31891)

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Although Sarcoma 180 (S-180) is not histocompatible with the random-bred HaICR Swiss mice, implants grow in 100% of these animals and regress in only 0 to 20% of the cases. This incidence of rejection is not increased even after the initial growth of the tumor is retarded by treatments with effective chemotherapeutic agents(1). Therefore, the complete regression of this tumor seen in Swiss mice fed a vitamin B_6 deficient diet(2), or treated with either 6-mercaptopurine (6 MP)(3) or kethoxal-bis (thiosemicarbazone) (KTS)(4), acquires particular significance.

It is not known whether the regression of S-180 induced by certain treatments is due to the action of host defenses directed against the chemotherapeutically impaired tumor. This idea is inconsistent with the fact that some of the treatments which induce complete regression of S-180 are also capable of depressing immunological defenses. For instance, both dietary depletion of vit. B_6 and treatment with 6 MP have been shown to impair certain immunological responses(5,6). Yet recent evidence indicated that in vit. B_6 deficient Swiss mice the regressions of S-180 are dependent upon the immunological competence of the host(7).

In this study it is shown that the incidence of tumor regressions elicited by KTS and 6 MP is greatly reduced in neonatally thymectomized mice. Although the initial growth inhibition of S-180 caused by these drugs was similar regardless of neonatal surgery, the delayed retardation of tumor growth appeared to be dependent upon the presence of the thymus. In intact mice, only a slight retardation of skin allograft rejection was caused by 6 MP and KTS at doses effective against S-180.

Materials and methods. The solid form of S-180 was implanted subcutaneously in HaICR Swiss mice. Standard procedures were followed for implantation of the tumor and evaluation of its growth(1). The purified diets used were described in detail elsewhere (5). Aqueous solutions of 6 MP were prepared immediately before use and were injected intraperitoneally once daily; KTS was fed mixed in the purified diets. Thymectomy

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TABLE I. Effects of Kethoxal-bis(Thiosemicarbazone) on Sarcoma 180 in Neonatally Thymectomized HaICR Swiss Mice.

	Treatment,* % in diet	No. of mice	8th day†		15th day†		10th week†		
			Δ Body wt, g‡	Avg tumor diam ± SD, mm	Δ Body wt, g‡	Avg tumor diam ± SD, mm	Mortality§		Survival
							All mice, %	Mice with tumor ≥11 mm, %	Tumor-free mice, %
Intact mice	None	43	+1.1	13.1 ± 3.8	−2.3	18.7 ± 4.5	95	95	5
	.05	19	−1.8	4.7 ± 1.2	+3.3	7.0 ± 4.6	74	74	26
	.1	59	−2.2	6.5 ± 5.5	− .8	7.8 ± 5.4	71	58	29
Thymectomized mice	.05	12	0	5.0 ± 1.0	+4.8	10.0 ± 4.5	92	92	8
	.1	38	−2.0	5.4 ± 2.4	− .6	12.2 ± 4.9	100	89	0
Splenectomized mice	.1	15	− .7	6.5 ± 2.9	+ .5	9.7 ± 7.3	53	53	47

* The drug was fed mixed in a complete purified diet for 7 days starting the day after tumor implantation.
† Counted from day of tumor implantation.
‡ Average change of body weight from that on day of tumor implantation.
§ Mortality evaluated according to size of tumor at last weekly measurement before death.

and splenectomy were performed 1 to 5 days after birth following standard procedures reported recently(7). Skin from C 57Bl 6/Ja female mice was grafted according to the Hauschka technique into female HaICR Swiss mice(8). In each experiment litter mates were distributed among the various groups and divided according to sex. At the end of the experiments all the thymectomized mice were autopsied and the thymus area was inspected visually with the aid of a dissecting microscope. In the doubtful cases histological study of the thymus area was performed; 5% of the animals had thymus fragments and were eliminated from the study.

Results. The data summarized in Table I show that at the end of a 7 day treatment with KTS, the growth of S-180 was markedly inhibited regardless of neonatal surgery. At the end of the second week, the average tumor diameter was larger in the neonatally thymectomized animals than in the other treated groups. In these experiments retardation of body growth and early mortality were minimal. Ten weeks after implantation complete tumor regression occurred in about 30% of the intact mice treated with KTS, in confirmation of data reported previously(4). The incidence of regression was increased in the neonatally splenectomized animals. In contrast, in the thymectomized mice the incidence of complete tumor regression was greatly reduced. In accordance with previous data(7), comparable results were obtained in mice subjected to surgery 1 to 5 days after birth. Thus, these data are grouped together. Mortality was evaluated according to the size reached by the tumor at the last weekly measurement preceding death. On the basis of experience in this laboratory, mortality was attributed to the growth of S-180 when the average diameter of the tumor was larger than 11 mm at the time of death. As shown in the Table, most of the animals which died had large tumors. Thus in this group of experiments death of thymectomized mice was attributed to causes other than tumor growth only in about 8% of the cases.

The growth of S-180 in mice treated with KTS is shown in Fig. 1. The results of a typical experiment were chosen as an example. To

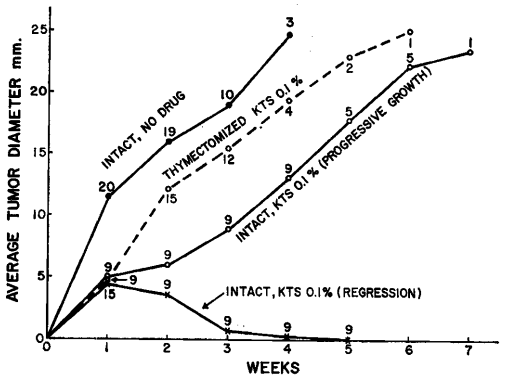

Fig. 1. Growth of Sarcoma 180 in intactand neonatally thymectomized Swiss mice treated with kethoxal-bis(thiosemicarbazone) (KTS). The number on top of each point represents number of mice surviving at time indicated.

evaluate the effects of host defenses on rates of growth of the tumors, the animals treated with KTS were grouped in the Figure according to the growth pattern of the tumors. It is apparent that initially the growth of S-180 was inhibited to the same extent in mice treated with the drug regardless of neonatal surgery and of whether the tumors would ultimately regress or would grow progressively. At the end of the second, third, and fourth week after implantation, however, the differences of average tumor diameter among the 3 groups of mice treated with KTS were statistically significant at the 5% level. The differences between the average diameter of tumors in untreated controls and in neonatally thymectomized mice treated with KTS were also significant at the same level of probability. The fate of S-180 in intact mice treated with KTS became evident only during the second week of tumor growth and was not related to any discernible difference of tumor inhibition observable at the end of treatment. Moreover, the delayed retardation of the growth of non-regressing tumors which occurred 2 to 4 weeks after implantation in intact mice previously treated with KTS appeared to be dependent not only upon the therapeutic treatment but also upon the immunological competence of the host. In fact, this retardation was significantly reduced in neonatally thymectomized animals.

At the end of a 7 day treatment with 6 MP the growth of S-180 was moderately inhibited regardless of neonatal surgery. At the end of the second week after tumor implantation, however, the size of S-180 in the intact and splenectomized mice treated with 6 MP was smaller than that in either the untreated controls or the thymectomized animals treated with the drug. As shown in Table II, ten weeks after implantation the incidence of complete regression of S-180 was increased in the intact mice treated with the drug. In contrast, in the neonatally thymectomized animals treated with 6 MP the incidence of tumor regression was even lower than that found in the untreated controls. This prevention of tumor regression was specifically related to neonatal thymectomy since it did not occur in neo-

TABLE II. Effects of 6-Mercaptopurine on Sarcoma 180 in Neonatally Thymectomized HaICR Swiss Mice.

			10th week†		
			Mortality‡		Survival
	Treatment, mg/kg/day*	No. of mice	All mice, %	Mice with tumor >11 mm, %	Tumor-free mice, %
Intact mice	None	47	81	81	19
	25	91	48	47	52
	50	33	58	55	42
Thymectomized mice	25	48	94	85	6
	50	27	93	78	7
Splenectomized mice	25	30	20	20	80
	50	29	21	21	79

* Intraperitoneal injections given once daily for 7 consecutive days starting the day after tumor implantation.

† Counted from day of tumor implantation.

‡ Mortality data evaluated according to size of tumor at last weekly measurement before death.

TABLE III. Effects of Neonatal Thymectomy on the Survival of C57Bl6/Ja Skin Grafts in HaICR Swiss Mice Treated with 6-Mercaptopurine or Kethoxal-bis(Thiosemicarbazone).

Surgery*	Treatment† mg/kg/day or % in diet	Scar formation, avg day ± SD‡	Time of scar formation‡ 6-10 days No./tot.	%	11-14 days No./tot.	%	15-19 days No./tot.	%	60th day‡ takes No./tot.	%
			6-Mercaptopurine							
None	None	8.4 ± 1.2	19/19	100					0/19	0
"	25 mg	8.5 ± .8	14/14	100					0/14	0
"	50 "	9.0 ± 1.2	13/15	84	15/15	100			0/15	0
Thy X	25 "	10.9 ± 3.0	7/17	41	13/17	76	15/17	88	2/17	12
"	50 "	12.5 ± 2.0	1/11	9	9/11	81	11/11	100	0/11	0
			Kethoxal-bis (Thiosemicarbazone)							
None	None	8.3 ± 1.3	9/9	100					0/9	0
"	.1%	13.1 ± 2.0	2/15	13	13/15	87	15/15	100	0/15	0
Thy X	.1%	14.8 ± 2.1	0/10	0	5/10	50	8/10	80	2/10	20

* Thymectomy was performed within 3 days from birth.
† Treatment was given for 7 days starting 2 days prior to skin grafting.
‡ Counted from day of skin grafting.

natally splenectomized mice. Indeed, in these animals the incidence of tumor regression was twice that observed in the intact mice treated with the drug. Most of the intact and splenectomized mice which died had large tumors. In contrast, about one fifth of the neonatally thymectomized mice probably died of toxicity, since they had tumors smaller than 11 mm in average diameter.

The fact that host defenses act against S-180 in mice treated with 6 MP is at variance with the known immunodepressant activity of this antimetabolite(6). Drugs like 6 MP, however, may inhibit the growth of S-180 selectively at doses which are not sufficient to impair the immunological responses of the host. The experiments summarized in Table III were carried out in order to assess this possibility. Skin graft survival was only slightly prolonged in intact mice treated with 6 MP or KTS at doses which in other experiments induced complete regression of S-180. In the neonatally thymectomized mice, however, both drugs substantially prolonged the survival of the skin grafts and induced 60 day "takes" with hair growth in a few cases. It is likely that neonatal thymectomy and treatment with either of the two drugs had synergistic effects in this respect.

Discussion. The therapeutic effects of 6 MP and KTS on S-180 have been reported(4,5). Information is now available which indicates the biochemical sites of inhibition probably responsible for the antiproliferative effects of 6 MP(9) and KTS(10,11,12). Regardless of the mechanisms of the antiproliferative action of these drugs, the basis for the delayed impairment and for the complete regression of S-180 caused by these agents is not clearly understood. In this and in other instances of tumor regression, it is important to evaluate the effects attributable to the immunological response of the host. The possibility that host defenses are responsible for the regression of the chemotherapeutically impaired tumor is in keeping with the expectation that random-bred Swiss mice react immunologically to a non-specific transplantable tumor. Yet the incidence of spontaneous rejection of S-180 is not increased after treatment with agents such as azaserine, 6-diaza-5-oxo-L-norleucine and N-methylformamide which effectively inhibit the initial growth of this tumor(1). The observation that the effects of 6 MP on a transplantable mouse mammary carcinoma was reduced by the administration of cortisone(13) suggested that the delayed therapeutic effect of this drug is mediated through defenses of the host. The results described herein provide direct evidence that the regression of S-180 observed in Swiss mice treated with 6 MP and KTS is dependent upon the immunological competence of the host.

The incidence of the regressions of S-180 induced by 6 MP and KTS was greatly reduced in neonatally thymectomized HaICR Swiss mice. Moreover, the delayed retardation of tumor growth caused by these drugs was

also reduced in these animals. In contrast, the initial inhibition of tumor growth seen at the end of treatment was comparable regardless of neonatal surgery. These observations are similar to those made when dietary vit. B_6 deficiency was the treatment responsible for the regression of S-180(7). In view of the well known neonatal role of the thymus in determining the capacity to develop immunological competence(16,17) in each of these cases, the conclusion can be drawn that the regression of the therapeutically impaired tumor is presumably brought about by immunological mechanisms of the host.

The causal relationship of the effects observed to neonatal thymectomy was strengthened by the fact that no reduction of the incidence of regression of S-180 was seen in neonatally splenectomized mice. In a previous study it was also shown that in vit. B_6 depleted mice the effects attributable to neonatal thymectomy did not occur in animals neonatally implanted with thymus autografts (7).

The incidence of regression induced by 6 MP and KTS was actually greater in the splenectomized than in the intact animals. The incidence of regression seen in vit. B_6 deficient mice was not significantly increased in neonatally splenectomized mice, however(7). The reason for this difference is not known. Recently, immunological enhancement of S-180 was elicited both in AKR(14) and in Swiss mice(15). In AKR mice this phenomenon was dependent in part upon the presence of the spleen(14). Therefore, it is possible that in neonatally splenectomized mice spontaneous immunological enhancement of S-180 is reduced. This would lead to an increased incidence of regression after treatment of the splenectomized animals with 6 MP and KTS. Vitamin B_6 deficiency may be able to reduce the spontaneous enhancement effectively in the intact mice. Hence, neonatal splenectomy would not yield a further therapeutic advantage.

The fact that the delayed antitumor effects of 6 MP and KTS are mediated through immunological reactions of the host is inconsistent with the known immunodepressant action of 6 MP(6) and with the observation that at relatively high doses KTS inhibits hemagglutinin responses in rats(18). At doses which are effective against S-180, however, 6 MP and KTS prolonged the survival of allogeneic skin grafts in intact Swiss mice only slightly (Table III). Therefore, the antitumor action of these drugs is selective enough to permit the occurrence of host immunological responses to the tumor. Thus a favorable balance seems possible between the effects of drugs against tumors and those against host defenses.

These and other data(7) imply that two phenomena are involved in the therapeutically induced regression of S-180 in HaICR Swiss mice. These are the initial inhibition of tumor growth caused by active and selective therapeutic treatments and the complete regression of the impaired tumor brought about by immunological responses of the host.

Summary. The incidence of complete regression of Sarcoma 180 induced by treatment with kethoxal-bis(thiosemicarbazone) and 6-mercaptopurine was greatly reduced in neonatally thymectomized but not in neonatally splenectomized animals. The inhibition of tumor growth observed at the end of treatment was not affected by neonatal surgery *per se*. In contrast, the delayel retardation of tumor growth seen in treated intact mice 2 to 4 weeks after implantation was significantly reduced in neonatally thymectomized animals. The survival of allogeneic skin grafts was only slightly prolonged in intact animals by treatment with the two drugs at doses capable of inducing therapeutic effects on Sarcoma 180. In neonatally thymectomized mice treated with the drugs, however, survival of the skin graft was substantially prolonged. These results indicate that, in intact animals implanted with Sarcoma 180, kethoxal-bis(thiosemicarbazone) and 6-mercaptopurine exert therapeutic effects by impairing the growth of the tumor selectively, thus permitting the immunological defenses of the host to bring about the complete regression of the chemotherapeutically impaired tumors.

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Role of the Carrier Protein in the Antibody Elicited to DNP Hapten.* (31892)

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The anti-hapten response of an animal immunized with a hapten-carrier conjugate is, to a large extent, influenced by the carrier molecule(1-4). It has been shown that the carrier not only influences the amount of anti-hapten antibody but the type and avidity of the immunoglobulin as well(5). The degree of conjugation of the hapten to the carrier and the genetic constitution of the immunized animal also markedly affect the anti-hapten response (6-8).

The present studies were designed to examine the relationship between the antigenicity of a carrier molecule and its effectiveness as a carrier. In order to keep variables such as the genetic constitution, sex, age, previous immunologic history of the immunized animals and the size and composition of the hapten-carrier complex as constant as possible, the following criteria were adopted: (a) The animals were to be highly inbred, of the same age, sex, and reared as identically as possible; (b) The carriers were to be proteins of the same size and function isolated from various species of animals including the animals to be immunized; and (c) The chemical structure of the hapten and number of haptenic groups attached onto each carrier were to be kept constant.

Reported below are studies of antibody elicited to the dinitrophenol (DNP) hapten in inbred mice immunized with different DNP-γ 2 globulin conjugates. The γ 2 globulin carriers were isolated from bovine, rabbit, rat and isologous mouse sera and the number of DNP groups per carrier molecule was the same for each conjugate.

Methods and materials. Preparation of carriers and hapten-carrier conjugates. Purified γ 2 globulin preparations of bovine (BGG), rabbit (RGG), and rat (RtGG) were obtained by DEAE-cellulose chromatography of commercially available fractions.‡ Purified mouse γ 2 globulin (MGG) was prepared by the method of Fahey and Horbett(9) using

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‡ BGG (Pentex lot #18); RGG (Pentex lot #36); and RtGG (Pentex lot #1064).